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Contribution of Lymphatically Transported Testosterone Undecanoate to the Systemic Exposure of Testosterone after Oral Administration of Two Andriol Formulations in Conscious Lymph Duct-Cannulated Dogs

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ABSTRACT

Orally administered testosterone (T) is ineffective in the treatment of male androgen deficiency syndromes due to extensive presystemic first-pass metabolism. In contrast, the lipophilic long-chain ester testosterone undecanoate (TU) exhibits androgenic activity that has been attributed to formation of T via systemic hydrolysis of lymphatically transported TU. However, there are no definitive data regarding the oral bioavailability of TU or the extent to which lymphatically transported TU contributes to the systemic availability of T after oral TU administration. This report describes the application of stable isotope methodology in a thoracic lymph duct-cannulated dog model to study the oral bioavailability and lymphatic transport of TU after postprandial administration. When administered as either Andriol or Andriol Testocaps, the mean (±S.E., $n = 4$) absolute bioavailability of TU was 3.25 ± 0.48 and 2.88 ± 0.88%, respectively, and lymphatically transported TU accounted for between 91.5 and 99.7% of the systemically available ester. Model-independent pharmacokinetic analysis indicated that 83.6 ± 1.6 and 84.1 ± 8.2% of the systemically available T, resulting from Andriol or Andriol Testocaps, respectively, was due to systemic hydrolysis of lymphatically transported TU. These data demonstrate that intestinal lymphatic transport of TU produces increased systemic exposure of T by avoiding the extensive first-pass effect responsible for the inactivation of T after oral administration.

The oral administration of testosterone (T) is ineffective in the treatment of male androgen deficiency syndromes because T is subject to almost quantitative presystemic first-pass metabolism mediated by the gut wall and liver (Daggett et al., 1978). Conversely, the lipophilic ester prodrug testosterone undecanoate (TU) demonstrates androgenic activity after oral administration to rats (Coert et al., 1975) and humans (Hirschhauser et al., 1975; Maisey et al., 1981; Skakkebaek et al., 1981). Because oral administration of TU results in the appearance of TU (and the metabolite 5α-dihydrotestosterone undecanoate; DHTU) in lymph of thoracic duct-cannulated rats (Coert et al., 1975; Noguchi et al., 1985) and humans (for whom a thoracic duct cannula was inserted after neck dissection surgery; Horst et al., 1976), the androgenic activity of orally administered TU is generally attributed to T (and 5α-dihydrotestosterone; DHT) formed during the systemic metabolic elimination of TU, which escaped the presystemic first-pass effect due to intestinal lymphatic absorption and transport (Coert et al., 1975; Horst et al., 1976).

Although it is accepted that lymphatic absorption of TU likely contributes to systemic T exposure after oral TU administration, the extent of that contribution has not been quantitatively determined. Indirect evidence of lymph transport of TU in humans was reported where the systemic exposure of T increased after oral TU administration in the fed state, compared with administration in the fasted state (Frey et al., 1979; Bagchus et al., 2003). However, human studies cannot determine the extent of lymphatic transport of TU, or the extent to which lymphatically transported TU leads to an increase in systemic T exposure, because it is not

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ABBREVIATIONS: T, testosterone; TU, testosterone undecanoate; DHTU, 5α-dihydrotestosterone undecanoate; DHT, 5α-dihydrotestosterone; LC, liquid chromatograph; LC-MS/MS, liquid chromatography coupled to tandem mass spectrometry; LC, liquid chromatography; LOQ, limits of quantitation; GC, gas chromatography; TG, triglyceride; AUC, area under the curve.

practical or possible to cannulate the thoracic lymph duct. Therefore, to characterize the absorption and bioavailability of orally administered TU, we have used a new lymph duct-cannulated dog model (Khoo et al., 2001) (a more representative model of the human than alternative animal models such as the rat; Edwards et al., 2001) to conclusively determine whether the intestinal lymphatics are responsible for the quantitative delivery of TU into the systemic circulation and to test the hypothesis that lymphatically transported TU is responsible for the majority of systemic T exposure after postprandial oral administration of TU. The current study used stable isotope methodology (to minimize intraindividual differences in drug distribution and elimination and provide the necessary data in a minimal number of dogs; Heck et al., 1979) to compare the relative performance of two commercially available TU formulations (Andriol and Andriol Testocaps) with respect to the amount of TU absorbed via the intestinal lymphatics and the resulting systemic exposure of T after oral administration of the TU formulations.

Materials and Methods

Materials. Deuterium-labeled TU ([^{2}H]-TU, batch DOFO0100A, labeled exclusively at C16 and C17) was supplied by Organon NV (Oss, The Netherlands) as a mixture of [^{2}H]$_3$-TU (79%), [^{2}H]$_2$-TU (20%), and [^{2}H]$_1$-TU (1%). Andriol (batch AN39477001), Andriol Testocaps (batch A39337001/387205), testosterone decanoate (TD), and methyl nortestosterone (MENT) were also supplied by Organon NV. Intralipid (10%) was purchased from Baxter Healthcare (Sydney, Australia) and Tri-Sil reagent was purchased from Pierce Chemical (Rockford, IL). All other chemicals were of analytical reagent grade and solvents were of HPLC grade. Water was obtained either from a Nanopure (Barnstead, Dubuque, IA) or Milli-Q (Millipore Corporation, Bedford, MA) water purification system.

Extemporaneous Preparation of an Intralipid Formulation of [^{2}H]-TU. [^{2}H]-TU for intravenous administration was incorporated into the Intralipid lipid emulsion by adaptation of a previous method (Humberstone et al., 1996). Briefly, 12 mg of [^{2}H]-TU (equivalent to 9.6 mg of [^{2}H]$_3$-TU) was dissolved in an *N*,*N*-dimethylacetamide/triacetin mixture [3:5 (v/v), 320 μl], and the resultant solution was added dropwise into 15 ml of Intralipid. Incorporation of [^{2}H]-TU into the emulsion droplets was achieved by 3 $\times$ 1-min periods of sonication with a probe sonicator (XL-Series operated at a power setting of 6; Misonix, Inc., Farmingdale, NY). During sonication, the temperature of the emulsion was kept below 10°C by cooling in a bucket of ice water. After the complete addition of [^{2}H]-TU, the concentration of [^{2}H]-TU in the emulsion was determined before and after centrifugation to determine the extent of incorporation of [^{2}H]-TU in the lipid droplets. The resulting emulsion was sterilized before administration by filtration through a sterile 0.22-μm filter. Immediately after administration in the dog study, a 50-μl aliquot of the filtered emulsion was collected and stored frozen at −80°C for determination of the exact [^{2}H]-TU concentration.

Oral Formulations of TU. The two TU formulations assessed in this study were Andriol and Andriol Testocaps. Andriol was formulated as soft gelatin capsules containing a 18.2% (w/w) solution of TU in oleic acid with the individual capsule composition being 40 mg of TU, oleic acid, gelatin, glycerol, Karion 83, sodium ethyl hydroxybenzoate, sodium propyl hydroxybenzoate, titanium dioxide (E171), and iron oxide red (E172). Andriol Testocaps soft gelatin capsules contained a 12.0% (w/w) solution of TU in lauroglycol FCC/castor oil [40:60% (w/w)] with the individual capsule composition being 40 mg of TU, lauroglycol FCC, castor oil, gelatin, glycerol, and sunset yellow (E110).

Surgical Procedures. All surgical and experimental procedures were approved by the local Institutional Animal Experimentation Ethics Committee. After induction of surgical anesthesia, the thoracic duct and portal vein of healthy adult female greyhound dogs (27–30 kg) was cannulated as described previously (Khoo et al., 2001). After surgery, the dogs were allowed to recover unrestrained for a period of 14 to 16 h, and during this time, they returned to normal ambulatory movement. An intravenous catheter was inserted into the cephalic vein before drug administration to enable serial sampling of peripheral blood during the study period. No attempt was made to control dogs with regard to their position relative to oestrus.

Experimental Procedures. Each dog was fed a standard can of commercial dog food (680 g) containing 5% crude fat (maximum) approximately 30 to 45 min before the intravenous administration of the [^{2}H]-TU/Intralipid emulsion, which was administered at a constant rate infusion (5.0 ml over 10 min) via the cephalic vein cannula. At the end of the infusion period, heparinized saline (2 ml) was rapidly flushed through the cannula to ensure complete delivery of the i.v. dose, and each dog was immediately administered an 80-mg oral dose of TU as either two Andriol capsules (n = 4 dogs) or two Andriol Testocaps capsules (n = 4 dogs). In addition to the dogs having access to drinking water ad libitum, 25 ml of normal saline was administered via the portal vein cannula at hourly intervals to limit any possible dehydration due to continuous collection of thoracic lymph.

Samples (5 ml) of systemic and portal blood were obtained simultaneously via the cephalic vein and portal vein cannulae, respectively, at the following times: −10 (i.e., predose), −5 (i.e., mid-infusion), 0 (i.e., end of infusion), 10, 20, 30, 40, 50, 60, 75, 90, 105, and 120 min, and every 30 min thereafter until 12 h postdose. Blood samples were collected into individual glass tubes containing potassium fluoride [50 μl of 10% (w/v) solution] as an inhibitor of nonspecific esterases. Once the blood had clotted, samples were centrifuged and the resultant serum stored at −80°C before analysis.

Lymph was collected continuously into 50-ml polypropylene tubes containing 75 mg of disodium EDTA (anticoagulant) for the 12-h postdosing period. All lymph samples collected per hour were pooled, and the total volume of lymph that had been collected was determined gravimetrically (assuming a specific gravity of 1 g/ml). Aliquots (50 μl) of the pooled lymph samples from each hourly collection period were dispensed into Eppendorf tubes and stored at −80°C before analysis.

Analytical Procedures. The techniques used to determine the concentration of labeled and unlabeled TU, T, DHT, and DHTU in serum and lymph, the concentration of TU in the intravenous formulation, and the concentration of triglyceride in lymph are described below.

Serum Concentrations of TU/DHTU. TU, [^{2}H]-TU, DHTU, [^{2}H]-DHTU, and the structurally related internal standard TD were isolated from serum by solid-phase extraction before analyte quantification by liquid chromatography coupled to mass spectrometry (LC-MS/MS) using electrospray ionization. Serum samples (200 μl) were mixed with internal standard solution (100 ng/ml in acetonitrile) and acetonitrile (70 μl) in glass tubes and transferred into SPE tubes (Bond Elut C$_{18}$; Varian Medical Systems, Palo Alto, CA), which had been preconditioned with methanol (3 ml) and water (3 ml) on a vacuum manifold. Samples were introduced dropwise onto the extraction column using minimal vacuum, and the column was then washed once with 3 ml of water. Analytes were slowly eluted from the column into a glass autosampler vial with acetonitrile/tetrahydrofuran [75:25 (v/v), 1.0 ml], and the eluate was then reduced to dryness within the autosampler vial using a vacuum centrifuge. The residue was reconstituted in acetonitrile before LC-MS/MS analysis. The LC system was a Hewlett Packard Series 1100 equipped with an LC analytical column (Supelcosil LC-8-DB, 50 mm $\times$ 4.6 mm, 5-μm particle size) running at a column temperature of 40°C. The flow was 0.5 ml/min and an 8-min gradient was used in which the mobile phase composition varied (linearly) from 10% water [0.2% (v/v) acetic acid] in methanol to 100% methanol over 6.9 min. The mass spec-

trometer was an API 3000 (Applied Biosystems, Foster City, CA) operated at a vaporizer temperature of 200°C and an ionization spray voltage of 5,500 V. In the MS/MS mode the protonated $[M + H]^+$ molecules of TU, $[^2H]_3$-TU, DHTU, $[^2H]_3$-DHTU, and TD were used as the precursor ion and measured as product ions at *m/z* ratios of 270.75, 274.09, 254.91, 258.15, and 270.86, respectively. The limits of quantitation (LOQ) for TU and DHTU (and the corresponding related labeled analytes) in serum were 0.5 and 1 ng/ml, respectively.

Lymph Concentrations of TU/DHTU. TU, [^{2}H]-TU, DHTU, [^{2}H]-DHTU, and the structurally related internal standard TD were isolated from lymph by liquid-liquid extraction before quantification by LC-MS/MS using electrospray ionization. Lymph samples (20 μl) were thoroughly mixed with internal standard solution (100 ng/ml in acetonitrile) and 0.1 M HCl solution (20 μl) in glass tubes. A 300-μl aliquot of 2-butanol was added with thorough mixing, after which the samples were centrifuged and the 2-butanol transferred to glass autosampler vials. The eluate was evaporated to dryness using a vacuum centrifuge, and the residue was reconstituted in acetonitrile (25 μl) before analysis. The LC analytical column (Chromolith SpeedROD RP18e, 50 mm × 4.6 mm; Merck, Darmstadt, Germany) was maintained at a column temperature of 40°C, and separations conducted at 1.00 ml/min where an 8-min gradient program was used in which the mobile phase composition remained constant [7% water, 0.2% (v/v) acetic acid in methanol] for 5 min, after which it changed to 100% methanol over 0.1 min where it remained for a further 0.9 min before returning to the starting conditions over a further 0.1 min. The mass spectrometer was an API 3000 (Applied Biosystems), and the operating conditions were identical to those for determination of TU in serum, except that the vaporizer temperature was set at 250°C. In the MS/MS mode, the protonated $[M + H]^+$ molecules of TU, $[^2H]_3$-TU, DHTU, $[^2H]_3$-DHTU, and TD were used as precursor ion and measured as product ions at *m/z* 271.10, 274.30, 255.20, 258.10, and 271.20, respectively. The LOQ for TU and DHTU (and the corresponding related labeled analytes) in lymph was 2 and 5 ng/ml, respectively.

Lymph and Serum Concentrations of T/DHT. T, [^{2}H]-T, DHT, [^{2}H]-DHT, and the structurally related internal standard MENT were isolated from dog serum and lymph samples by solid phase extraction. The isolated compounds were converted to their trimethylsilyl (TMS) derivatives, extracted with *n*-hexane, and quantified using capillary gas chromatography (GC) coupled with mass spectrometry detection in the positive ion chemical ionization mode. Serum (200 μl) and lymph (25 μl) samples were mixed with internal standard solution (50 ng/ml in acetonitrile) and 10% acetonitrile in 0.08 M HCl solution (200 and 450 μl for serum and lymph, respectively) in glass tubes. After a 30-min equilibration period, the solutions were transferred into SPE tubes [Isolute C_{18} extraction column (Separtis, Grenzach-Wyhlen, Germany), preconditioned with methanol (1 ml) and water (1 ml)] on a vacuum manifold. Samples were introduced onto the extraction columns and each column was washed twice with 1 ml of water. Extraction columns were then placed in centrifuge tubes and centrifuged (4 min at 3500 rpm) before eluting each column with 1 ml of methanol into a 4-ml glass tube. The eluate was evaporated to dryness using a vacuum centrifuge, 25 μl of Tri-Sil derivatizing reagent was added, and the tubes were then mixed and allowed to stand for 15 min at 60°C. Water (0.5 ml) and *n*-hexane (1.2 ml) were then added, and after thorough vortex mixing and centrifugation, the *n*-hexane layer was transferred into a glass vial before evaporation to dryness using a vacuum centrifuge. The residue was reconstituted in acetonitrile and an aliquot injected onto the GC. The GC system was a HP 5890 Series II gas chromatograph equipped with a fused-silica capillary column (DB-17, 20 m × 0.18 mm, 0.30-μm film thickness; Agilent Technologies, Palo Alto, CA). Helium was the carrier gas at a column head pressure of 200 kPa, and the injector and transfer line temperatures were set at 260 and 300°C, respectively. A 7-min program was used where the oven temperature remained constant for 1 min before increasing linearly (25.0°C/min) to 300°C over the subsequent 6-min period. The mass spectrometer was a MAT SSQ-7000 (Thermo Finnigan, San Jose, CA) operated with an electron energy of −70 V, an emission current of 200 μA, and an ion source temperature of 200°C. The TMS derivatives of T, DHT, $[^2H]_3$-T, and $[^2H]_3$-DHT were measured at *m/z* 361.0, 363.0, 364.0, and 366.0, respectively, whereas the TMS derivative of MENT was measured at *m/z* 361.0. The LOQ of T and DHT in serum (and the corresponding related labeled analytes) were 0.2 and 0.4 ng/ml, respectively, whereas the corresponding values in lymph were 2 and 4 ng/ml.

[^{2}H]-TU in Intralipid. A 50-μl aliquot of the Intralipid emulsion ($n = 5$) was diluted in propan-2-ol (950 μl), and the total TU concentration of the resulting solution was determined using an HPLC system consisting of a Spectra System P4000 pump, Spectra System AS3000 autosampler, and Spectra System UV2000 UV detector equipped with an LC analytical column (Kromasil C_{18}, 100 mm × 4.6 mm, 5-μm particle size; Varian Medical Systems) operated at a column temperature of 30°C. The mobile phase was 100% methanol flowing at 1.0 ml/min, the injection volume was 10 μl, and the detection wavelength was 254 nm. The concentration of $[^2H]_3$-TU in the Intralipid emulsion was equivalent to 79% of the total TU concentration.

Acceptance Criteria for HPLC and GC Assays. Each analytical run included blank serum or lymph samples, duplicate calibration samples, authentic study samples, and triplicate quality control samples at three different concentrations spanning the calibration range. The results of each analytical run were accepted when the correlation coefficient of the calibration curve exceeded 0.99, when at least two-thirds of the calibration standards were accepted and the accuracy of all accepted calibration points was between 80 and 120%, and when the accuracy of at least two-thirds of the QC samples at each concentration was between 80 and 120%.

Lymph Concentrations of Triglyceride. After a 1 in 10 dilution of lymph samples in Milli-Q water, triglyceride (TG) concentrations were determined using a standardized clinical chemistry analyzer (Roche Cobas Mira, Basel, Switzerland) and a commercial enzyme-based colorimetric assay kit (Roche Diagnostics, Mannheim, Germany).

Data Analysis. The mass of analyte transported into thoracic lymph during each sampling period was calculated as the product of the analyte concentration and total volume of lymph collected during the sampling period. The fraction of the dose transported (F_{lymph}) was calculated as the ratio of cumulative mass of analyte transported and oral dose.

Analysis of Serum Pharmacokinetic Profiles. The maximum serum concentration ($C_{\max}$), and the time to reach the maximum ($T_{\max}$) were noted directly from the individual profiles. The area under the serum concentration-time profiles from time 0 up to the last measured concentration ($AUC^{0-t_{\text{last}}}$) was calculated by WinNonLin version 4 (Pharsight, Apex, NC) using the linear trapezoidal method. Where the apparent terminal phase of the serum concentration-time profiles was evident (i.e., for [^{2}H]-TU and [^{2}H]-T), the area obtained by extrapolation to infinite time was added to AUC^{0-t}_{last} to obtain the area from time 0 to infinity $AUC^{0-\infty}$. Serum clearance of [^{2}H]-TU ($CL_{[^2H]\text{-TU}}$) was calculated as the ratio of intravenous dose and $AUC^{0-\infty}$, i.e.,

$$CL_{[^2H]\text{-TU}} = D_{[^2H]-TU}/AUC^{0-\infty}_{[^2H]-TU} \quad (1)$$

The proportion of the dose absorbed into the systemic circulation via the portal route ($F_{\text{portal blood}}$) was calculated from the ratio of the dose-normalized AUC values for TU and [^{2}H]-TU, i.e.,

$$F_{\text{portal blood}} = (AUC^{0-t_{\text{last}}}_{TU}/D_{TU}) \cdot (D_{[^2H]-TU}/AUC^{0-\infty}_{[^2H]-TU}) \cdot 100\% \quad (2)$$

The proportion of the dose absorbed into the systemic circulation via the thoracic lymph (F_{lymph}) was calculated from the ratio of the cumulative mass of TU transported in lymph and the dose of orally administered TU, i.e.,

F_{lymph} = Cumulative mass of TU transported in lymph/D_{TU} (3)

The predicted systemic serum AUC for TU due to lymphatically transported TU ($AUC_{TU}^{\text{lymph derived}}$) was calculated as follows:

$$AUC_{TU}^{\text{lymph derived}} = \text{cumulative mass of TU transported in lymph}/CL_{[^2H]\text{-TU}} \quad (4)$$

where it was assumed that 1) there was no isotope effect, i.e., TU behaves in an identical manner to [^{2}H]-TU in vivo; 2) TU entering the systemic circulation via the lymph is subject to the same dispositional and elimination events as [^{2}H]-TU administered i.v. in Intralipid; and 3) that the pharmacokinetics of TU and related compounds were linear.

The predicted systemic exposure to T, due to lymphatically transported TU ($AUC_T^{\text{lymph derived}}$), was calculated as follows:

$$AUC_T^{\text{lymph derived}} = AUC_{TU}^{\text{lymph derived}} \cdot (AUC_{[^2H]\text{-T}}^{0-\infty}/AUC_{[^2H]\text{-TU}}^{0-\infty}) \quad (5)$$

where it was assumed that the factors determining the systemic exposure to T due to lymphatically transported TU are identical to those determining the systemic exposure to [^{2}H]-T after intravenous administration of [^{2}H]-TU.

Statistical Analysis. Statistically significant differences in pharmacokinetic parameters and lymph transport arising from administration of the Andriol or Andriol Testocaps formulations were analyzed by Student's t test at a significance level of $\alpha = 0.05$.

Results

Systemic Serum Concentrations after i.v. Administration of [^{2}H]-TU. Fig. 1 presents the mean systemic serum concentration versus time profiles for [^{2}H]-TU, [^{2}H]-DHTU, [^{2}H]-T, and [^{2}H]-DHT after i.v. administration of [^{2}H]-TU to thoracic lymph duct-cannulated dogs, and the calculated pharmacokinetic parameters are given in Table 1. Pharmacokinetic parameters were not calculated for [^{2}H]-DHTU because there were no measurable serum concentrations in five of the eight dogs. After i.v. administration of the same [^{2}H]-TU formulation to each dog, there was no statistically significant difference ($p > 0.05$) in any calculated i.v. pharmacokinetic parameter between the groups of dogs that simultaneously received oral administration of Andriol or Andriol Testocaps (Table 1). Consequently, the mean value for each parameter determined after i.v. administration was calculated using data from all eight dogs.

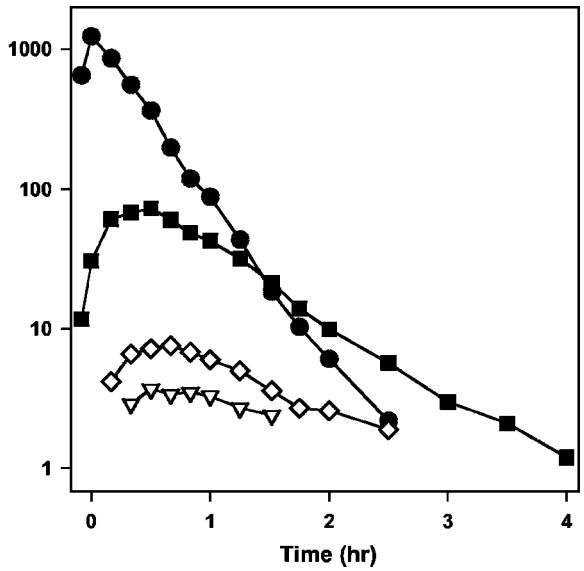

Fig. 1. Mean (n = 8) systemic serum concentrations (nanomolar) of [^{2}H]-TU (●), [^{2}H]-DHTU (▽), [^{2}H]-T (■), and [^{2}H]-DHT (◇) after i.v. administration of [^{2}H]-TU to postprandial lymph duct-cannulated dogs as a 10-min infusion in Intralipid. Data are presented as the mean of all study dogs as there was no statistically significant difference in the i.v. pharmacokinetic parameters for groups of dogs receiving either oral Andriol or Andriol Testocaps. S.E. error bars are within the size of the individual symbols.

Maximum serum concentrations of [^{2}H]TU were observed at the end of the infusion period (i.e., $T_{max} = 0$) with maximum concentrations of [^{2}H]-T and [^{2}H]-DHT being observed at later times with the mean T_{max} values of 26 ± 3 and 49 ± 6 min for [^{2}H]-T and [^{2}H]-DHT, respectively. The mean C_{max} value for [^{2}H]-TU was 1,234 ± 65 nM, with lower values observed for [^{2}H]-T (77.7 ± 4.1 nM) and [^{2}H]-DHT (7.8 ± 1.1 nM). In the apparent terminal phase, serum concentrations of [^{2}H]-TU declined with a $t_{1/2}$ approximately 2-fold shorter than that of [^{2}H]-T and [^{2}H]-DHT, the mean $t_{1/2}$ values being 15.8 ± 0.5, 32.1 ± 2.7, and 37.5 ± 5.8 min for [^{2}H]-TU, [^{2}H]-T, and [^{2}H]-DHT, respectively. The calculated $AUC^{0-\infty}$ values were 36,429 ± 2,024, 5,466 ± 264, and 589 ± 99 nM · min for [^{2}H]-TU, [^{2}H]-T, and [^{2}H]-DHT, respectively. The mean clearance (CL) and V_D values of [^{2}H]-TU were 0.222 ± 0.012 l/min and 5.08 ± 0.31 liters, respectively.

Lymphatic Transport and Portal Concentrations after Oral Administration of TU. The mean rate of lymphatic transport of TG, TU, and DHTU into thoracic lymph after postprandial oral administration of 80 mg of TU as Andriol or Andriol Testocaps is presented in Fig. 2, A to C, together with the mean cumulative recovery of each compound in thoracic lymph (Fig. 2, D–F). Lymph concentrations of T and DHT were below the LOQ (or where measurable, so low as to be considered negligible) for the entire sampling period. The maximum rate of lymphatic TU transport was observed during the 1- to 2-h postdosing period, whereas the corresponding maxima for TG and DHTU transport occurred during the 2- to 3-h postdosing period. Recovery of TG in thoracic lymph corresponded to >80% of the administered mass of lipid confirming the integrity of each cannulated dog. In terms of drug transport, the cumulative recoveries of TU and DHTU in thoracic lymph accounted for 3 and 0.12% of orally administered TU, respectively. The mean portal serum concentration versus time profiles of TU, T, and DHT are presented in Fig. 3. Portal serum concentrations for DHTU are not available because the concentrations were below the LOQ (i.e., <1 ng/ml) for the entire sampling period in five of the eight dogs.

Systemic Serum Concentrations of TU, DHTU, T, and DHT. The mean systemic serum concentration versus time profiles of TU, T, and DHT after postprandial oral administration of 80 mg of TU as Andriol or Andriol Testocaps to thoracic lymph duct-cannulated dogs are presented in Fig. 4. Profiles for DHTU are not presented as systemic serum DHTU concentrations were below the LOQ for the entire sampling period in six of the eight dogs. Pharmacokinetic parameters determined for TU and T in each dog are presented in Table 2, together with the mean values for all dogs receiving the same oral formulation. There was considerable intersubject variability in the systemic C_{max}, T_{max}, and AUC values for TU and T, which gained access to the systemic circulation via the portal blood (i.e., in the lymph cannulated dogs). There was no statistically significant difference ($p >$

TABLE 1
Mean (± S.E.) systemic serum pharmacokinetic parameters for [^{2}H]-TU, [^{2}H]-T, and [^{2}H]-DHT determined after a 10-min i.v. infusion (3.91 mg) of [^{2}H]-TU to dogs that simultaneously received an oral dose of 80 mg of TU administered as either Andriol (n = 4) or Andriol Testocaps (n = 4)

Compound	i.v. Parameter	Oral Andriol Group (n = 4)	Oral Andriol Testocaps Group (n = 4)	p Value[a]	Combined i.v. Data[b] (n = 8)
[^{2}H]-TU	C_{max} (nM)	1,250 ± 116	1,217 ± 77	0.81	1,234 ± 65
	$AUC^{0-\infty}$ (nM · min)	36,137 ± 3,835	36,720 ± 2,089	0.90	36,429 ± 2,024
	$t_{1/2}$ (min)	15.2 ± 0.7	16.4 ± 0.7	0.25	15.8 ± 0.5
	CL (l/min)	0.228 ± 0.022	0.218 ± 0.012	0.70	0.222 ± 0.012
	V_D (l)	4.98 ± 0.50	5.17 ± 0.44	0.78	5.08 ± 0.31
[^{2}H]-T	C_{max} (nM)	74.3 ± 6.5	81.1 ± 5.3	0.44	77.7 ± 4.1
	t_{max} (min)	25 ± 5	26 ± 4	0.90	26 ± 3
	$AUC^{0-\infty}$ (nM · min)	5,187 ± 452	5,746 ± 261	0.33	5,466 ± 264
	$t_{1/2}$ (min)	32.3 ± 4.5	31.8 ± 3.5	0.93	32.1 ± 2.7
[^{2}H]-DHT	C_{max} (nM)	6.3 ± 1.3	9.3 ± 1.4	0.16	7.8 ± 1.1
	t_{max} (min)	42 ± 11	37 ± 6	0.68	49 ± 6
	$AUC^{0-\infty}$ (nM · min)	418 ± 73	760 ± 145	0.08	589 ± 99
	$t_{1/2}$ (min)	38.7 ± 11.7	36.3 ± 4.7	0.86	37.5 ± 5.8

[a] p values are the result of the two-tailed Student's t test of the mean parameter estimates between the two formulations.
[b] The mean ± S.E. values for all study dogs (n = 8) was calculated because there was no statistically significant difference ($p > 0.05$) between Andriol and Andriol Testocaps for any of the measured parameters.

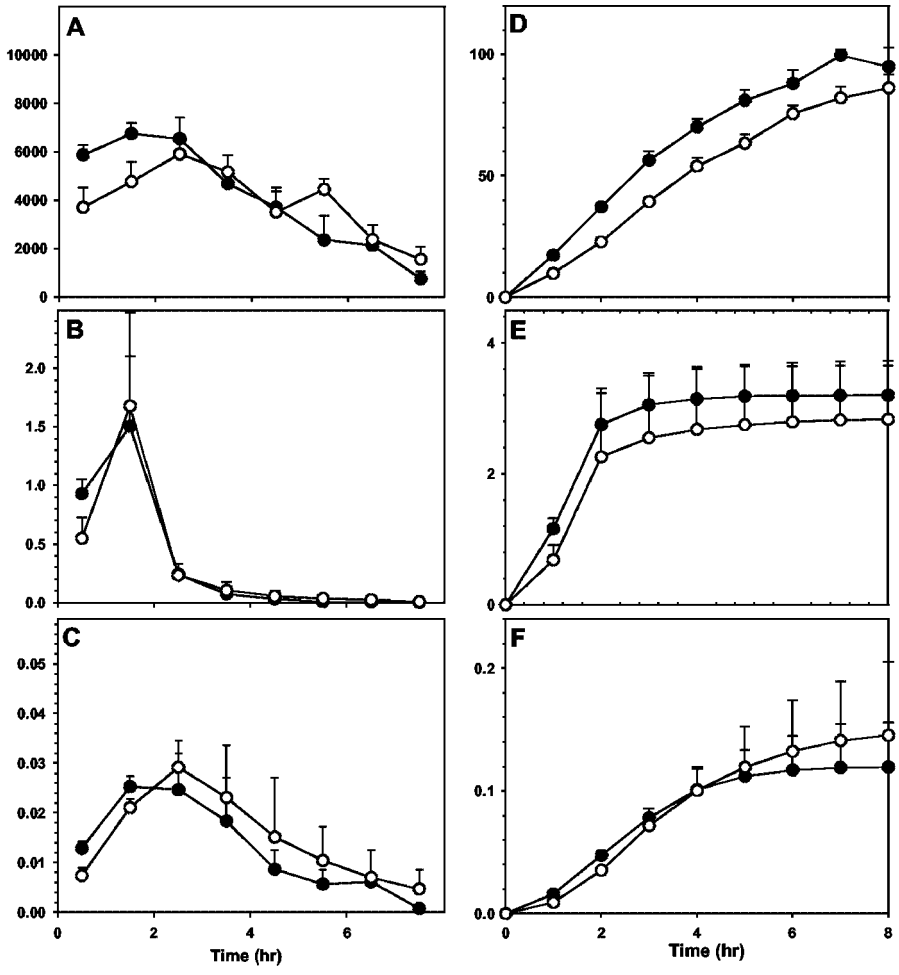

Fig. 2. Mean (± S.E.) rate (milligrams per hour) and cumulative (percentage of dose) thoracic lymphatic transport of TG (A and D), TU (B and E), and DHTU (C and F) after oral administration of 80 mg of TU to postprandial lymph duct-cannulated dogs as either Andriol (closed symbols, n = 4) or Andriol Testocaps (open symbols, n = 4).

0.05) in any pharmacokinetic parameter in the Andriol or Andriol Testocaps groups.

Systemic Exposure of TU and T. The mean contribution of intestinal lymphatic transport and portal blood absorption to the systemic availability (expressed as a percentage of the oral dose) of TU administered as Andriol or Andriol Testocaps is presented in Table 3. Irrespective of the administered formulation, the lymphatic route was responsible for the majority of systemic TU exposure (>95%), even though the total mass of TU reaching the systemic circulation represented approximately 3% of the orally administered TU. Andriol and Andriol Testocaps were indistinguishable in terms of either the total oral TU availability or the fractional contributions of the two absorption routes to oral availability. The contribution of lymphatically derived T (i.e., T resulting from lymphatically absorbed TU) and portally absorbed T to

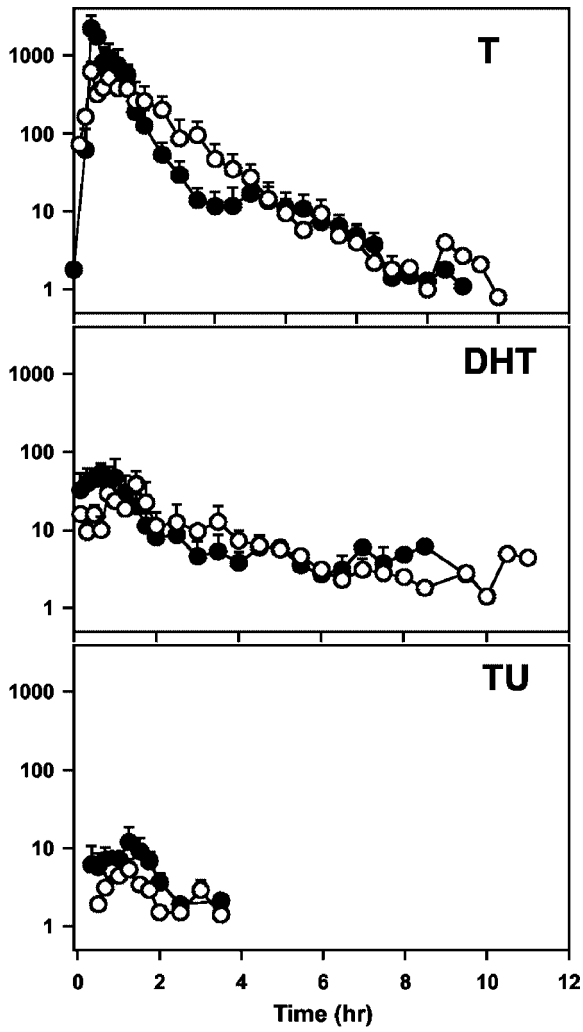

Fig. 3. Mean (± S.E.) portal serum concentrations (nanomolar) of T, DHT, and TU after oral administration of 80 mg of TU to postprandial lymph duct-cannulated dogs as either Andriol (closed symbols) or Andriol Testocaps (open symbols).

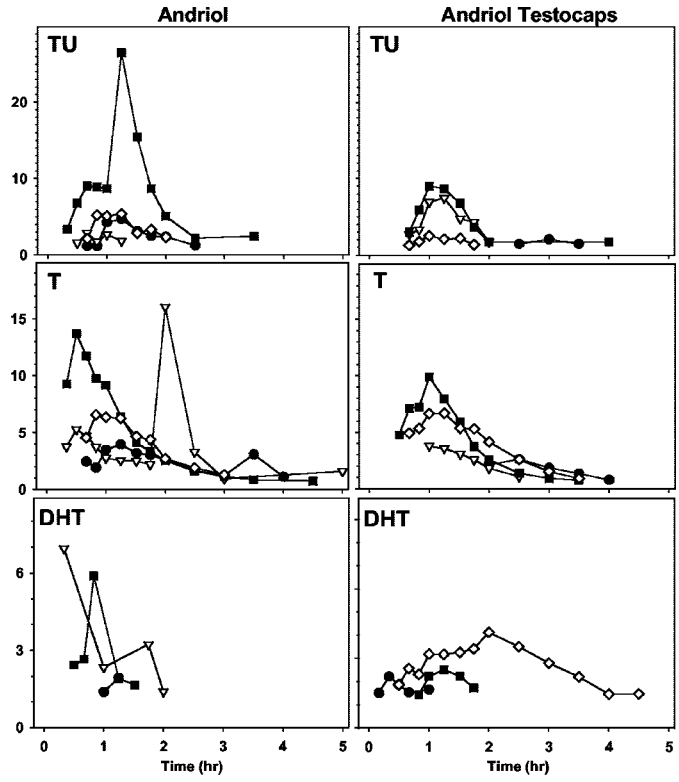

Fig. 4. Systemic serum concentration (nanomolar) versus time profiles of TU, T, and DHT in individual lymph duct cannulated dogs after postprandial oral administration of 80 mg of TU as either Andriol (dog 2, ●); dog 4, ▽; dog 5, ■; and dog 7, ◇) or Andriol Testocaps (dog 1, ●; dog 3, ▽; dog 6, ■; and dog 8, ◇). Data have been omitted where analyte concentrations were below the LOQ.

systemic T exposure is presented for each dog in Table 4 as the lymph derived and measured systemic AUC values of T, respectively (i.e., $AUC^{lymph\ derived}$ and $AUC^{measured}$, respectively). For all dogs, lymph-derived T constituted the major proportion of systemically available T, and there was no statistically significant difference ($p > 0.05$) in the total systemic exposure to T (where exposure is evaluated on the basis of AUC^{total}) between Andriol and Andriol Testocaps (Table 4). Although the mean $AUC^{measured}$ of T after administration of Andriol Testocaps was approximately 30% lower compared with Andriol (i.e., T arising from portal blood absorption), the difference was not statistically significant ($p > 0.05$) and the absolute values are of minor relevance because the predicted systemic T exposure arising from lymphatically derived TU (which accounts for approximately 85% of total systemic T exposure) was similar for the two formulations.

Discussion

Testosterone undecanoate exhibits androgenic activity after oral administration in 1975 (Coert et al., 1975; Hirschhauser et al., 1975). Oral administration of TU results in the lymphatic appearance of TU and DHTU in rat (Coert et al., 1975; Noguchi et al., 1985) and humans (Horst et al., 1976), and it is generally accepted that the androgenic activity originates from T and DHT formed after systemic hydrolysis. Both TU and DHTU avoid the hepatic first-pass effect by absorption via the lymphatics (Coert et al., 1975; Horst et al., 1976). Over the past 25 years, there have been numerous reports of the efficacy and safety of orally administered TU in T replacement therapy (Luisi and Franchi, 1980; O'Carroll et al., 1985; Carani et al., 1990; Mårin et al., 1992; Gooren, 1994; Morales et al., 1997; Geurts and Coelingh Bennink, 2000); however, few studies have attempted to characterize efficacy in terms of T bioavailability due to TU administration. Although some studies have attempted such an assessment, they have not provided direct evidence of the contribution of lymphatic TU to increased systemic T exposure. For example, Frey et al. (1979) compared the relative bioavailabilities of orally administered T (in the form micronized T, crystalline TU, and TU in arachis oil) and reported an increase in systemic T exposure when TU was administered in conjunction with a high-fat meal. Although this result is consistent with enhanced lymphatic drug transport due to the simultaneous absorption of dietary lipids (Geurts and Coelingh Bennink, 2000; Khoo et al., 2001, 2002; Bagchus et al., 2003), the data are not conclusive nor quantitatively insightful. Similarly, Tauber et al. (1986) reported that the mean absolute bioavailability of T after oral administration of TU to women was 6.83 ± 3.32%, whereas the mean absolute bioavailability of orally administered T after oral T ad-

TABLE 2
Systemic serum pharmacokinetic parameters for testosterone undecanoate and testosterone determined in lymph duct cannulated dogs following oral post-prandial administration of 80 mg of TU as either Andriol or Andriol Testocaps

Formulation	Dog Number	Testosterone Undecanoate[a]			Testosterone[a]		
		C_{max}	t_{max}	$AUC^{0-t_{last}}$	C_{max}	t_{max}	$AUC^{0-t_{last}}$
		nM	*min*	*nM · min*	*nM*	*min*	*nM · min*
Andriol	2	4.69	74	305	3.95	84	493
	4	2.85	40	108	16.05	130	792
	5	26.49	75.25	1434	13.69	40.5	968
	7	5.39	77.5	326	6.55	60.75	550
	Mean ± SE	9.86 ± 5.57	67 ± 9	543 ± 301	10.06 ± 2.86	69 ± 19	701 ± 110
Andriol Testocaps	1	2.05	183	127	2.6	162	239
	3	7.47	76	412	3.78	70	246
	6	9.02	60	715	9.88	70	723
	8	2.5	61.25	134	6.69	85	677
	Mean ± S.E.	5.26 ± 1.75	95 ± 30	347 ± 139	5.74 ± 1.63	87 ± 22	471 ± 132

[a] No significant difference ($p > 0.05$) in any parameter between Andriol and Andriol Testocaps.

TABLE 3
The systemic availability of TU expressed as a percentage of the administered dose (mean ± S.E., $n = 4$) arising from lymphatic transport (F_{lymph}) and portal blood transport ($F_{portal\ blood}$), and as the total transport (F_{total}) in thoracic duct cannulated dogs after postprandial administration of 80 mg of TU as either Andriol ($n = 4$) or Andriol Testocaps ($n = 4$)

Availability of TU[a]	Andriol	Andriol Testocaps
F_{lymph} (% dose)	3.20 ± 0.46	2.85 ± 0.89
$F_{portal\ blood}$ (% dose)	0.054 ± 0.029	0.036 ± 0.015
F_{total} (% dose)	3.25 ± 0.48	2.88 ± 0.88

[a] No significant difference ($p > 0.05$) between any parameter.

ministration was 3.64 ± 2.45%. Again, these results suggest that TU leads to increased systemic T exposure (relative to oral T administration); however, they do not conclusively demonstrate the role of lymphatic TU absorption because they do not determine the relative contributions of the lymphatic and portal absorption pathways.

To determine the contribution of lymphatic and portal blood absorption to the systemic bioavailability of highly lipophilic drugs, our laboratory developed and validated a thoracic lymph duct-cannulated dog model (Khoo et al., 2001). The current study was undertaken to determine the contribution of intestinal lymphatic transport of TU to systemic availability after postprandial administration of two TU formulations (Andriol or Andriol Testocaps) and to examine the hypothesis that lymphatic TU transport provides an advantage in oral T therapy because it provides for the majority of systemic T exposure after postprandial administration.

Systemic Serum Concentrations after i.v. Administration of [^{2}H]-TU. After the i.v. infusion, serum concentrations of [^{2}H]-TU declined rapidly in a monoexponential manner with a mean half-life of 15.8 ± 0.5 min with the concomitant formation of [^{2}H]-DHTU, [^{2}H]-T, and [^{2}H]-DHT arising from the respective hydrolysis and/or reduction of [^{2}H]-TU due to systemic esterase and 5α-reductase activity. Because the serum clearance of the relevant TU metabolites was not individually determined in this study, it was not possible to determine the fractional conversion of [^{2}H]-TU to [^{2}H]-DHTU, [^{2}H]-T, and [^{2}H]-DHT. However, the data qualitatively confirm that the therapeutic benefit derived from TU is likely mediated by T and DHT.

The low variability in the serum concentration versus time profiles of [^{2}H]-TU, [^{2}H]-T, and [^{2}H]-DHT after i.v. administration of [^{2}H]-TU indicated excellent intersubject consistency in the systemic conversion of [^{2}H]-TU to [^{2}H]-T and [^{2}H]-DHT (Fig. 1). Furthermore, there was no significant difference in the i.v. profiles for the two groups that received the different oral formulations, indicating that compositional differences in the oral TU formulations did not affect the systemic pharmacokinetics of TU (Table 1).

Absorption into Lymph after Oral TU Administration. TU and DHTU were observed in thoracic lymph after oral administration of TU to postprandial lymph duct-cannulated dogs, consistent with previous reports in rats (Coert et al., 1975; Noguchi et al., 1985) and humans (Horst et al., 1976). The profile of TU transport in lymph was essentially the same after oral administration of either the Andriol or Andriol Testocaps formulations (Fig. 2, B and E). Although of minor qualitative importance in terms of the mass transported, the profile of lymphatic TU transport was different compared with that observed for TG and DHTU transport. For example, although the maximum rate of lymphatic TU transport occurred 1 to 2 h postdosing, the maximal rate of transport of TG and DHTU occurred 2 to 3 h postdosing (Fig. 2, A and C). Furthermore, the rate of lymphatic TU transport declined rapidly after reaching its maximal transport rate, whereas the rate of TG and DHTU transport declined more gradually (Fig. 2, A–C). These profiles provide possible insight into the disposition of TU (and DHTU) within the gastrointestinal tract and enterocyte before absorption into lymph. For example, because TU is metabolized within the intestinal lumen and gut wall (Coert et al., 1975; Horst et al., 1976), which is the basis for the high serum concentrations of T and DHT observed in portal blood in the present study (Fig. 3), the change in the relative rates of TG and TU transport after the 1- to 2-h postdosing period likely reflects the reduction in the available mass of TU within the enterocyte for incorporation into chylomicrons. If a limiting factor in the kinetics of TU lymph transport was rapid hydrolysis within the intestinal lumen, then the observation of the prolonged transport of DHTU in concert with TG transport suggests that DHTU (formed by 5α -reductase metabolism of TU) may have a longer residence time within the enterocyte lipid-processing microdomains which could arise from either differences in the metabolic stability or enterocyte-based processing mechanisms for DHTU and TU.

TABLE 4
The contribution of lymphatically transported TU to the total systemic exposure of T following postprandial oral administration of 80 mg of TU as either Andriol or Andriol Testocaps

Formulation	Dog Number	Testosterone Undecanoate	Testosterone			Percentage of Contribution of Lymphatically Transported TU to the AUC^{total} for T
		$AUC^{lymph\ derived}$[a]	$AUC^{lymph\ derived}$	$AUC^{measured}$	AUC^{total}	
		nM · min	*nM · min*	*nM · min*	*nM · min*	
Andriol	2	22,217	2,016	493	2,509	80.3
	4	22,067	4,035	792	4,827	83.6
	5	36,330	4,516	968	5,474	82.4
	7	19,338	4,054	550	4,604	88.1
	Mean ± S.E.	24,988 ± 3,838	3,655 ± 558	701 ± 110	4,354 ± 642	83.6 ± 1.6[b]
Andriol Testocaps	1	14,650	2,544	239	2,787	91.4
	3	26,025	4,652	246	4,898	95.0
	6	7,696	1,073	723	1,796	59.7
	8	46,321	6,453	677	7,130	90.5
	Mean ± S.E.	23,673 ± 8,442	3,681 ± 1,180	471 ± 132	4,153 ± 1,185	84.1 ± 8.2[b]

[a] The $AUC^{lymph\ derived}$ value for TU was used to determine the $AUC^{lymph\ derived}$ value for T according to eq. 4.
[b] No significant difference ($p > 0.05$) in any parameter between Andriol and Andriol Testocaps.

Contribution of Lymphatic and Blood Absorption to Systemic Availability of TU. In the intact animal, thoracic duct lymph empties directly into the systemic circulation at the junction of the internal jugular and brachiocephalic vein. Therefore, the cumulative mass of drug recovered in thoracic lymph during the experimental period is equivalent to the mass of drug otherwise systemically available after absorption via the lymphatic route. In the lymph duct cannulated dog, the apparent bioavailability calculated from the ratio of the dose normalized systemic serum AUC values after oral and i.v. administration (determined using a stable TU isotope, which is not expected to exhibit an in vivo isotope effect; Baba et al., 1979, 1980; Shinohara et al., 1980, 1988; Fujioka et al., 1986, 1989; Shinohara and Baba, 1990) is equivalent to the fraction of the dose systemically available after absorption via the portal vein.

Based on the ratio of the dose-normalized serum AUC values for TU and $[^2H]_3$-TU, the mean fraction of the TU dose reaching the systemic circulation via the portal vein was similar and extremely low at 0.054 ± 0.029 and 0.036 ± 0.015% from Andriol and Andriol Testocaps, respectively (Table 3). In terms of the lymphatic transport, the mean fraction of the orally administered dose of TU recovered in thoracic lymph was 3.20 ± 0.46 and 2.85 ± 089% from Andriol and Andriol Testocaps, respectively (Table 3). There was no statistical difference ($p > 0.05$) in the extent of portal blood or lymphatic transport of TU after administration of either Andriol or Andriol Testocaps.

When the total systemic TU availability is calculated as the sum of the availabilities due to portal and lymphatic TU absorption (i.e., $F_{portal\ blood} + F_{lymph}$), it is obvious that lymphatic absorption was responsible for 93.0 to 99.8% of the TU that ultimately reaches the systemic circulation (Table 3). Because the total systemic availability of TU after postprandial administration of Andriol (3.25 ± 0.48% of the dose) was not significantly different ($p > 0.05$) compared with Andriol Testocaps (2.88 ± 0.88% of the dose), the two formulations are indistinguishable in terms of their systemic TU exposure.

Factors Contributing to Systemic T Exposure. It is evident from Fig. 1 that $[^2H]$-TU delivered into the systemic circulation is rapidly converted to $[^2H]$-T. However, it was not possible to estimate the fractional conversion of $[^2H]$-TU to $[^2H]$-T without an estimate of the serum clearance of $[^2H]$-T and this precluded calculation of the mass of T reaching the systemic circulation as a consequence of lymphatic transport of TU. This was addressed by using eqs. 4 and 5 to calculate a value for the term $AUC_T^{lymph\ derived}$ to estimate the systemic exposure of T arising from lymphatic transport of TU (i.e., the systemic exposure of T arising from the contents of the thoracic duct emptying into the systemic circulation).

After postprandial administration of 80 mg of TU as Andriol, the mean value of $AUC_T^{lymph\ derived}$ was 4,354 ± 642 nM · min and the corresponding value for Andriol Testocaps was 4,153 ± 1,185 nM · min (Table 4). Because the mean systemic T serum AUC values ($AUC^{measured}$) after administration of Andriol and Andriol Testocaps were 701 ± 110 and 471 ± 132 nM · min, respectively (Table 4), it is clear that T derived from systemic hydrolysis of lymphatically absorbed TU was the major contributor to the total systemic T exposure (i.e., $AUC_T^{lymph\ derived} + AUC^{measured}$). In addition, there was no statistically significant difference ($p > 0.05$) in AUC^{total} between dogs administered Andriol or Andriol Testocaps (Table 4). Consequently, the two formulations were indistinguishable in terms of the systemic exposure to T.

Conclusion

Numerous strategies (typically involving administration of 17-α-alkylated testosterone prodrugs via the oral and injected routes) have previously been applied to circumvent the fact that direct oral T administration is ineffective in androgen replacement therapy. However, orally administered testosterone undecanoate is both safe and efficacious upon oral drug administration (Luisi and Franchi, 1980; O'Carroll et al., 1985; Carani et al., 1990; Mårin et al., 1992; Gooren, 1994; Morales et al., 1997; Geurts and Coelingh Bennink, 2000). The results from the current study demonstrate conclusively that the therapeutic advantage associated with oral TU administration is a consequence of lymphatic transport of TU to the systemic circulation. Furthermore, the study demonstrated conclusively that there is no difference in the systemic exposure of T, or TU, resulting from postprandial oral administration of Andriol or Andriol Testocaps.

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