The Plasma Pharmacokinetics of R-(+)-Lipoic Acid Administered as Sodium R-(+)-Lipoate to Healthy Human Subjects.

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Abstract

BACKGROUND: The racemic mixture, RS-(+/-)-alpha-lipoic acid (rac-LA) has been utilized clinically and in a variety of disease models. Rac-LA and the natural form, R-lipoic acid (RLA), are widely available as nutritional supplements, marketed as antioxidants. Rac-LA sodium salt (NaLA) or rac-LA potassium salt (KLA) has been used to improve the aqueous solubility of LA. STUDY RATIONALE: Several *in vitro* and animal models of aging and age-related diseases have demonstrated efficacy for the oral solutions of LA salts in normalizing age-related changes to those of young animals. Other models and studies have demonstrated the superiority of RLA, the naturally occurring isomer over rac-LA. Despite this, RLA pharmacokinetics (PK) is not fully characterized in humans, and it is unknown whether the concentrations utilized in animal models can be achieved *in vivo*. Due to its tendency to polymerize, RLA is relatively unstable and suffers poor aqueous solubility, leading to poor absorption and low bioavailability. A preliminary study demonstrated the stability and bioavailability were improved by converting RLA to its sodium salt (NaRLA) and pre-dissolving it in water. The current study extends earlier findings from this laboratory and presents PK data for the 600-mg oral dosing of 12 healthy adult subjects given NaRLA. In addition, the effect of three consecutive doses was tested on a single subject relative to a one-time dosing in the same subject to determine whether plasma maximum concentration (Cmax) and the area under the plasma concentration versus time curve (AUC) values were comparable to those in animal studies and those achievable via intravenous infusions in humans. METHODS: Plasma RLA was separated from protein by a modification of a published method. Standard curves were generated from spiking known concentrations of RLA dissolved in ethanol and diluted in phosphate-buffered saline (PBS) into each individual's baseline plasma to account for inter-individual differences in protein binding and to prevent denaturing of plasma proteins. Plasma RLA content was determined by the percent recovery using high-performance liquid chromatography (electrochemical/coulometric detection) (HPLC/ECD). RESULTS: As anticipated from the preliminary study, NaRLA is less prone to polymerization, completely soluble in water, and displays significantly higher Cmax and AUC values and decreased time to maximum concentration (Tmax) and T1/2 values than RLA or rac-LA. In order to significantly extend Cmax and AUC, it is possible to administer three 600-mg RLA doses (as NaRLA) at 15-minute intervals to achieve plasma concentrations similar to those from a slow (20-minute) infusion of LA. This is the first study to report negligible unbound RLA even at the highest achievable plasma concentrations. (*Altern Med Rev* 2007;12(4):343-351)

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Introduction

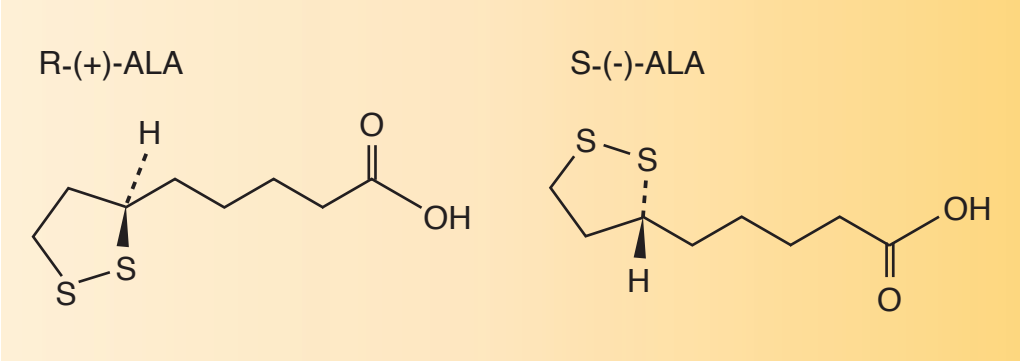
The racemic mixture of alpha-lipoic acid, RS-(+/-)-alpha-lipoic acid (rac-LA), has been utilized clinically and in therapeutic applications such as amanita mushroom poisoning,[1] diabetic neuropathy,[2] metabolic syndrome,[3] burning mouth syndrome,[4,5] peripheral artery disease,[6] and treatment of a variety of skin[7] and liver diseases.[8-10] Rac-LA and the natural form, R-lipoic acid (RLA), are widely available as nutritional supplements marketed as dietary antioxidants.[11,12] Based on positive outcomes in various models, RLA has been recommended for the prevention and treatment of both Alzheimer's[13] and Parkinson's[14] diseases.

Structurally, lipoic acid (LA) is a medium chain (C_8) fatty acid with sulfur atoms at C_6 and C_8. Because the C_6 carbon is chiral, LA exists as two enantiomers or stereoisomers: R-(+)-lipoic acid and S-(-)-lipoic acid (SLA) (Figure 1). It is well established that salts of weak acids (pKa LA(COOH)=4.76-5.3)[15] have higher aqueous solubilities and absorption than the respective free acids.[16] Therefore, if a relatively hydrophobic drug or nutrient can be given as a salt, its solubility can be increased and dissolution and absorption should be improved. Although the bioavailability of RLA is limited, that of NaRLA should be enhanced. RLA salts are reconverted to the free acid in the acidic pH of the stomach, where rapid absorption begins. Subsequently, the free acid is more extensively absorbed in the proximal portion of the duodenum; whereas, free LA is more slowly and less efficiently absorbed during the slower transit through the more alkaline pH of the intestines.[17,18] Alkali metal salts (sodium, potassium), alkaline earth salts (barium, calcium, and magnesium), amino acid salts (arginine, lysine, methionine, creatine), natural amines/amides (nicotinamide, pyridoxamine), and synthetic amine salts such as tromethamine (tris or 2-amino-2-(hydroxymethyl) propane-1, 3-diol) of LA have all been synthesized. Except for the tromethamine salt, however, PK values in humans are unknown.[17]

All known biological systems employing LA exclusively utilize RLA.[19] Although SLA (and SLA-containing products, e.g., rac-LA) is generally considered to be non-toxic, its presence in medicines and dietary supplements is due solely to achiral manufacturing processes. Despite lack of evidence of overt toxicity from *in vitro* and animal models, there are indications that SLA can antagonize the activity of a specific marker improved by RLA or function as a competitive inhibitor of RLA.[20-28]

The individual LA enantiomers (RLA, SLA) and rac-LA appear to be pharmacologically distinct,[17,23,28-33] with unique PK profiles.[17,34] Although not fully characterized *in vivo*, there are indications exogenously administered RLA is the eutomeric form (the physiologically and therapeutically superior isomer) of LA for animal and human uses.[11,17,35] Despite

Figure 1. Molecular Diagrams of R-(+)-Lipoic Acid and S-(-)-Lipoic Acid



Three dimensional molecular diagrams depicting both the absolute configurations (R and S), which are mirror images, and the reversed optical activities (+) and (-), which are useful in conceptualizing how one enantiomer can react differently than its mirror image with a receptor, transporter, signaling molecule, protein, or enzyme in vivo. Only R-(+)-lipoic acid is naturally occurring and is the eutomeric form of lipoic acid.

the superiority of RLA in these models, the basic PK parameters of the individual enantiomers and the corresponding alkali metal salts have not been characterized in humans.

Reported PK values for the single enantiomers of LA are surprisingly rare. A comprehensive literature search revealed eight published references mentioning widely varying human plasma maximum concentration (Cmax) values (0.4 and 1.15 mcg/mL per 1000 mg) for enantiomerically pure RLA[34,36-39] or Cmax of 5 and 10 mcg/mL (~25-50 μM) for 300 and 600 mg RLA as the tromethamine salt.[17,19,40] Only a thesis, review article, and patent by the same investigators provide the same partial concentration versus time curves for a single subject.[34,37,38] A human pilot trial utilizing 604-mg tablets containing RLA tromethamine salt has been conducted and reported in a doctoral thesis, but it has not been submitted for peer review or publication.[17] To date, there are no reports of any human PK values with SLA (as the single enantiomer).

Hermann et al demonstrated increased bioavailability of 200 mg rac-LA administered as a predissolved salt in aqueous solution.[41] This trial also demonstrated PK values were approximately 50-percent higher for RLA than SLA.[42] Krone demonstrated increased Cmax and area under the curve (AUC) values in rats when RLA was administered in the form of an oral solution without SLA,[17] indicating that SLA may interfere with the absorption of oral RLA. In the present study, improved Cmax and bioavailabilities (measured as AUC) for the NaRLA dosage form were anticipated.

Material, Methods, and Study Design

Chemicals and Reagents

The sodium salt of RLA was synthesized and validated by GeroNova Research, Inc. (Richmond, CA). HPLC analysis was performed using the USP method for rac-LA. The RLA starting material and the NaRLA were analyzed by chromatography, polarimetry, mass spectroscopy (MS), and nuclear magnetic resonance to determine chemical and enantiomeric purities (both greater than 99.5 percent). RLA reference standards were manufactured and purified, and the enantiomeric purities were validated by chiral HPLC/MS and polarimetry and used throughout the study as the analytical standards. Analytical reagent grade acetonitrile, methanol, and phosphoric acid were purchased from GFS Chemicals (Columbus, OH). Aqueous solutions were prepared using reverse osmosis-treated and distilled water (≥18MΩ). Control human plasma for optimization of LA extraction and recovery experiments was purchased from Bioreclamation Inc., Hicksville, NY.

Equipment and Chromatography

A preliminary study in this laboratory utilizing HPLC/ECD revealed plasma levels of RLA from baseline (~0.05 mcg/mL) up to 45 mcg/mL (~225 μM) could be rapidly detected and quantified.[43] A Hewlett Packard (HP 1050 series HPLC system) coupled with an HP auto-sampler was used for chromatographic separations. RLA in standards, plasma extracts, and spiked-plasma extracts was determined by the method of Sen et al.[44] RLA was separated on Phenomenex Gemini (Torrance, CA) and Vydac (purchased from National Analytical Instruments; San Rafael, CA) C18 analytical columns (4.6 x 250 mm) at ambient temperature. The mobile phase was 50 mM phosphate buffer:acetonitrile:methanol (50:30:20), pH 2.7, with a flow rate of 1 mL/min. RLA was detected using an ESA Coulochem II multi-electrode detector (ESA, Inc; Chelmsford, MA) set as follows: guard cell, 0.90 mV; electrode 1, 0.40 mV; electrode 2, 0.85 mV. Data was acquired using HP ChemStation software.

Standard Solutions and Calibration Curves

Stock standard solutions of RLA were prepared in ethanol-water (1:1) at 10 mg/mL and stored at -20° C. For LC system calibration, appropriate working stock dilutions were made fresh daily (100, 50, 10, 5, 1, 0.1, 0.01 mcg/mL) and injected into the liquid chromatography (LC) system. After system calibration with authentic RLA standards, RLA plasma extracts were quantified relative to RLA curves determined from RLA recovered from each subject's baseline plasma. Plasma was spiked with 0.5, 1, 10, and 50 mcg/mL of RLA (final concentrations).

Subjects, Treatments, Sample Preparation

Subjects gave informed, written consent prior to the study. Subjects were not taking prescription drugs or being treated for medical conditions. Seven of 12 subjects were already regular users of NaRLA or a mixture of RLA and R-dihydrolipoic acid (R-DHLA) and followed personalized daily vitamin and nutritional supplement programs. Based on a previous trial showing that rac-LA does not accumulate or modify subsequent PK profiles, a three-day washout period for RLA was considered adequate.[45] Subjects were instructed to avoid consumption of alcohol or nutritional supplements for three days prior to the trial. Blood glucose was checked at each time point in the NaRLA trials using the Onetouch Ultra glucose monitor.

Subjects consumed 600 mg RLA (as NaRLA, based on the RLA content) dissolved in 200 mL purified water 3-4 hours after a light meal to reduce the incidence of nausea. A preliminary study revealed this had no adverse affect on plasma levels of RLA (data not shown).

Table 1. Subjects: Physical Data

	n	Mean BMI	Mean age	Mean blood glucose (mg/dL)
Women	4	20.7	48.3	92.3
Men	8	24.6	45.1	94.7
All subjects	12	23.3	46.2	93.5

In addition to the one 600-mg dose, subject 3 received three 600-mg doses RLA (as NaRLA) at t=0, 15, and 30 minutes, after a two-week washout period and subsequent to the initial 600-mg dose in order to determine the effect of three doses on Cmax and AUC relative to a single dose in the same subject.

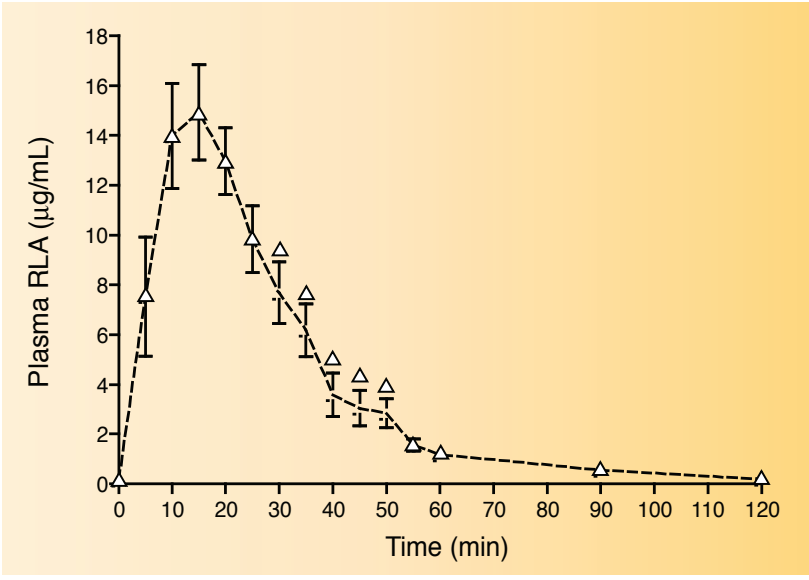
Whole blood samples were collected into 9-mL evacuated sodium or lithium heparin tubes from 12 subjects (Table 1) at various time points (0-60 minutes, every 5 minutes, and then every 30 minutes out to 90 or 120 minutes). Samples were chilled for 25 minutes in ice water (-4° C) to prevent hemolysis and centrifuged at 4,000 RPM for 12 minutes.

Plasma was separated and each subject's baseline plasma was used to make calibration standards. Unbound (free) RLA levels were determined immediately. The remainder of the plasma sample was frozen until use. Triplicate extractions of each plasma sample were prepared and duplicate analysis performed by LC. Blood glucose was monitored at each time point.

Extraction and Recovery

Several literature methods of isolating RLA and R-DHLA from plasma proteins were evaluated and tested for extraction/recovery efficiency.[17,43,46,47] Free RLA was separated from plasma using Amicon Centrifree micro-partition filter cartridges (molecular weight cutoff, 30,000).[48] Total RLA was extracted from plasma samples by vortexing plasma samples in LC mobile phase, incubation for 15 minutes at 37-40° C, followed by centrifugation to pellet precipitated proteins. The supernatant was used for LC analysis. To generate standard curves that accounted for individual variations in RLA plasma protein binding, RLA standards were spiked into each subject's baseline plasma sample at various concentrations (see above). Appropriate dilution of the RLA working stocks were made in phosphate-buffered saline (PBS) solution to prevent ethanol-induced artifactual alteration of spiked RLA binding to plasma proteins. Validated RLA reference standards were dissolved in ethanol-water (1:1) and then further diluted in PBS before spiking into plasma. Final ethanol concentrations introduced to the plasma were kept below 0.01 percent. The spiked plasma samples were vortexed for 1 minute at 37° C and allowed to cool at room temperature for 15 minutes before extraction with mobile phase, in parallel with the unknown plasma samples. Extraction efficiency of RLA spiked into baseline plasma samples (0.5-50 mcg/mL) ranged from 84-96 percent using this method. Baseline plasma spike-extraction sample sets were made for each individual and used to quantify RLA in the unknown plasma samples of each subject.

Figure 2. Plasma Pharmacokinetics for all Subjects (n=12)



Data shown are averaged plasma RLA values for each time point in the study.

Pharmacokinetic Analysis

Plasma RLA Cmax and the corresponding time to maximum concentration (Tmax) were obtained from the raw data. PK Solutions software (Montrose, CO), which estimates PK parameters using noncompartmental analysis, was utilized for subsequent data calculations. AUC was calculated using trapezoidal summation and is a representation of the relative bioavailability of the analyte. The plasma half-life (T1/2) of the terminal elimination phase was calculated based on the data from Tmax to the last quantifiable time point.

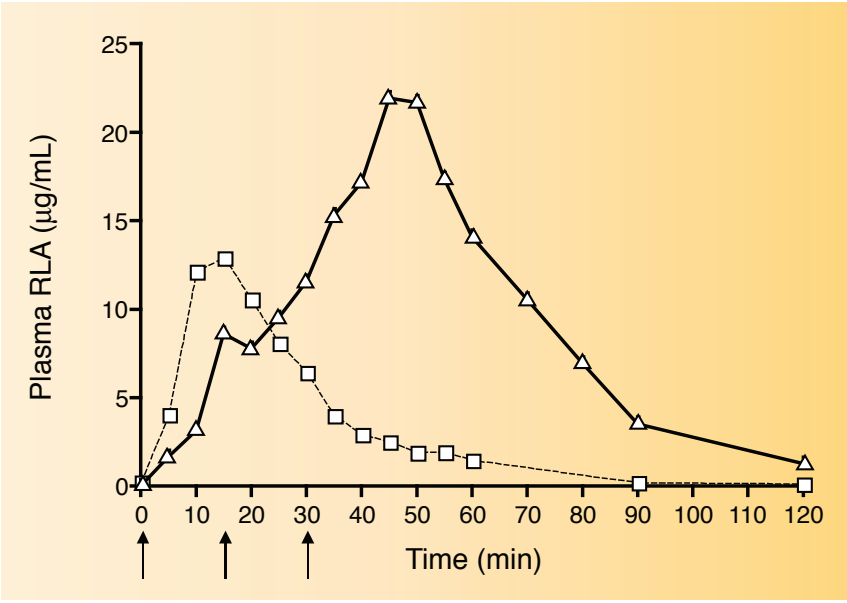
Results

Baseline levels of RLA (Limit of Detection ~50-250 ng/mL) could be detected by liberation from plasma proteins with mobile phase using a modification of the method of Chen et al.[46] Although previous studies indicate rac-LA does not accumulate in blood or tissues,[17] in the present study baseline RLA was detected only in subjects who are regular users of a mixture of RLA and R-DHLA, even subsequent to a three-day washout period at levels of 0.05-0.25 mcg/mL. Analysis of the plasma concentration-time curves in a preliminary study with two subjects revealed that 600 mg pure RLA yields low Cmax and bioavailability (as measured by AUC) values, significantly lower than an equivalent weight of rac-LA.[43] The preliminary study provided the first published PK values for NaRLA. The Cmax and AUC values for NaRLA in the male subject were 25.86 and 3.3 times higher, respectively, than RLA. In the female subject, NaRLA produced Cmax and AUC values 17.9 and 2.67 times higher, respectively, than pure RLA.[43] RLA (100 mg; commonly found in nutritional supplements) is barely distinguishable from baseline,

Table 2. Pharmacokinetic Parameters of 600-mg Oral Dose R-(α)-Lipoic Acid Sodium Salt (based on RLA content) in Human Subjects

Subject	Gender	BMI	Cmax (µg/mL)	Tmax (min)	AUC (µg·min/mL)	AUC (µg·h/mL)	$t_{1/2}$ (e-phase)
1	m	31.0	18.0	15	508.4	8.473	16.6
2	f	23.5	14.7	10	438.5	7.308	17.7
3	m	28.0	12.9	15	376.2	6.270	15.7
4	f	19.6	16.1	10	350.9	5.848	5.2
5	m	25.7	12.2	15	406.3	6.772	13.6
6	m	24.1	33.8	10	804.1	13.40	9.6
7	m	20.3	13.6	10	350.4	5.840	13.3
8	f	21.8	15.2	20	452.0	7.533	13.2
9	m	24.1	21.0	20	684.1	11.40	11.8
10	m	21.4	13.3	20	395.4	6.590	11.3
11	m	22.5	11.0	15	297.2	4.953	15.7
12	f	17.8	10.6	10	235.6	3.927	24.1
	PK parameter stats						
	Mean		**16.0**	**14**	**441.6**	**7.36**	**14.0**
	Median		14.2	15	400.9	6.68	13.5
	S.D.		6.32	4.2	160.2	2.67	4.66

Figure 3. Plasma Pharmacokinetics for a Single Subject



Subject 3 was given three sequential 600-mg doses at t=0, 15, 30 min (indicated by arrows). The individual's single 600-mg dose PK curve is presented for comparison (dashed line/boxes). A two-week washout period lapsed between the two dose regimens.

presumably due to poor absorption (data not shown). As expected, use of RLA as the pre-dissolved sodium salt resulted in significant increases in Cmax and AUC in all 12 subjects (Figure 2; Table 2). The average dose was 8.25 mg/kg, generating a mean Cmax of 16.03 mcg/mL (range: 10.6-33.8 mcg/mL), median Tmax of 15 minutes (range: 10-20 minutes), and mean AUC of 441.59 mcg min/mL (7.36 mcg hr/mL). The plasma concentration time profile had negligible effect on plasma glucose levels measured at each time point. Subject 3 consumed three 600-mg doses of RLA (as NaRLA) (Figure 3), resulting in a Cmax of 21.9 mcg/mL, AUC of 1,049 mcg min/mL (17.48 mcg x hr/mL), and extended the Tmax out to 45 minutes. An unexpected finding of this study was that, at plasma concentrations as high as 30 mcg/mL (~150 µM) (Cmax subject 6), negligible free RLA was detected.

Discussion

Although the clinical significance of baseline RLA (0.05-0.25 mcg/mL) is not fully characterized, previous trials have correlated low baseline RLA with a variety of disease states.[7,10,49-52] It has been suggested that the presence of RLA in plasma may function to maintain the plasma redox status, which shifts to a more oxidized state with age and in numerous diseases.[53,54] The current study and previous findings from this laboratory conclude that pure RLA is not suitable for use in nutraceutical or pharmaceutical products. Rather, it should be treated as raw material for further processing into stable, bioavailable dosage forms. PK data reveals pure RLA is significantly less bioavailable than RLA found as a 50-percent component of rac-LA; and RLA in a salt form is considerably more bioavailable than an equivalent dose of rac-LA (RLA + SLA). This indicates SLA may function as a competitive inhibitor in the absorption of RLA.

Different forms of RLA produce dramatically different PK values. Recently, RLA was compared to NaRLA in humans using a simple crossover design.[43] This study compared Cmax and AUC values of a pre-dissolved aqueous solution containing 600 mg RLA (as NaRLA) to those of 600 mg RLA in the same subjects. In a single male (subject 1), NaRLA produced Cmax of 14.1 mcg/mL; whereas, RLA resulted in a Cmax of 0.7 mcg/mL (increase of 25.86x). The AUC in the same subject was 5.18 mcg hr/mL for NaRLA versus 1.56 mcg hr/mL for RLA (increase of 3.3x). In a single female (subject 4), Cmax was 18.1 mcg/mL for NaRLA versus 1.01 mcg/mL for RLA (increase of 17.9x) and the AUC was 5.71 mcg hr/mL for NaRLA compared to 2.14 mcg hr/mL for RLA (increase of 2.67x).

The data shows Cmax and relative bioavailability as measured by AUC for the aqueous solution of three doses of 600 mg RLA (as NaRLA) taken at 15-minute intervals is similar to those reported for a

20-minute I.V. infusion of 300 mg rac-LA.[55] Cmax and AUC values were significantly increased over the one-time dose. Cmax values reached 21.9 mcg/mL versus 12.9 mcg/mL and AUC values increased from 376.2 mcg min/mL (6.27 mcg hr/mL) to 1,049 mcg min/mL (17.48 mcg min/mL).

Based on the mean values from eight human PK studies utilizing 600 mg rac-LA, the current authors suggest the threshold of activation of the therapeutic effects of LA is equal to Cmax of 4-5 mcg/mL (~20-25 μM) and AUC equal to 2.85 mcg hr/mL.[17,45,46,55-59] More consistent therapeutic results may be achieved at plasma concentrations of 10-20 mcg/mL (~50-100 μM) of the natural enantiomer, RLA.[17,60] The upper limit of the human therapeutic concentration range is ~50 mcg/mL (~250 μM).[41]

A basic principle of pharmacology states that free drug (not bound to plasma proteins) is more biologically active than plasma protein-bound drug and mostly responsible for the therapeutic action.[61-63] Most PK studies and assays for LA have measured the total LA content in serum or plasma rather than differentiating the concentrations of free and bound LA. Many different techniques with varying degrees of efficiency have been utilized to determine the total LA concentrations. *In vitro*, rac-LA spiked into human plasma is not measurable in "free" form until plasma protein binding is saturated at ~4-5 μM (0.825-1.030 mcg/mL).[64] In rats, it was reported only 20 percent (~0.8 mcg/mL) of the total plasma RLA (~4 mcg/mL) was free (using the Centrisart ultrafiltration device). The level of free/unbound RLA was concentration independent and temperature dependent. The "free" value corresponded to the amounts of RLA found in skeletal muscle by microdialysis and was extrapolated to account for plasma protein binding in humans.[17] Since the therapeutic efficacy of a compound is associated with the "free" levels and levels of "free" drug concentration differ from species to species (and even wide inter-individual differences are known), the decision was made to test the levels of "free" RLA in each subject's plasma.

This is the first study to demonstrate negligible amounts of "free" RLA, even at Cmax. This indicates that a re-assessment of a fundamental principle of pharmacology (i.e., the therapeutically active form of a drug or nutrient is correlated to the amount of "free" versus bound drug) relative to the mechanisms of transport and action of LA is necessary.

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