"Lipoic acid–biotin mandate"

Human Lipoic Acid Use: Is Supplemental Biotin Necessary?

A Critical Re-Evaluation

**Question:** “Several companies selling R-lipoic acid or alpha lipoic acid claim it is essential to take supplemental biotin with lipoic acid products. Why doesn’t GeroNova add biotin to their products? Since biotin is cheap and might be beneficial, why don’t you recommend people take it with lipoic acid?”

**Answer:** I call this issue the "lipoic acid–biotin mandate". Scientists, doctors and companies making claims for the necessity of supplemental biotin with lipoic acid use made this determination after reading the conclusions of a single study by Zempleni et al.¹ issuing strong warnings to lipoic acid users with inherited carboxylase (the biotin-dependent enzymes) deficiencies that was I believe inappropriately extrapolated to the general lipoic acid using public. This article is frequently cited and is the first reference to appear on the lipoic acid page on encyclopedia.com.

Surprisingly, Zempleni et al did NOT recommend supplemental biotin either for people with or without carboxylase deficiencies. I speculate on the possible reasons for this omission below.

The questionable methodology and conclusions of this study raise questions concerning its relevance to humans and until now has not been critically examined. The authors used a rat model that is not readily extrapolated to humans, based their conclusions on a reporting error in lipoic acid pharmacokinetic (PK) data and did not take into consideration the differences in dosage forms, routes of administration and the differences between lipoic acid and biotin PK in rats and humans.

I plan to send my critical analysis of the study to the authors and the Journal of Nutrition (which published the initial study) for comment. Below is a highlight of my reasons for removing d-biotin from our products as well as the Zempleni et al study, findings, conclusions and my critical analysis.

GeroNova Research was to my knowledge the first company to add pure d- (+)-biotin (the natural, optically active form) to our R- (+)-lipoic acid products. I decided to remove it and continue to question the necessity of supplemental biotin for the reasons cited below:

1-Difficulty in obtaining homogenous blends between lipoic acid and biotin on the industrial scale.

R-(+) lipoic acid or racemic (+/-) alpha lipoic acid capsules or tablets usually contain 100-300 mg active but biotin is used in the microgram range (100-300 ug) range, 300-1000 times less. This poses a formidable challenge for supplement manufacturers to blend the two together on a multi-kilo scale and to end up consistently with an even blend and to obtain the stated label claim. I learned this the "old fashioned way"... by doing it and analyzing the mixtures.

2-Regular users of lipoic acid products **MIGHT** benefit from supplemental d-biotin or d,l-biotin but it is probably wiser to consume biotin at a different time than lipoic acid to insure optimal absorption of both nutrients. It has been demonstrated by several groups of researchers (including Zempleni et al) in this and other studies that lipoic acid, biotin and pantothenic acid utilize at least one of the same transport systems and co-administration results in competition for uptake in various model systems.¹-⁴ This finding challenges the wisdom of combining lipoic acid and biotin in the same oral dosage form or even consuming the two nutrients concurrently.

**Brief Review of the Zempleni et al Study Design**

For the complete Zempleni et al experimental design, results and conclusions please view here:

Zempleni et al used 5 groups of rats all receiving intraperitoneal (i.p.) injections of each of the following for 28 days; a control group receiving i.p. injection of a phosphatidyl choline (PC) dispersion in saline, a low dose lipoic acid group; a high dose lipoic acid group, a high dose lipoic acid + biotin, and a group receiving hexanoic acid (a structural analog of lipoic acid). The test compounds were all administered as PC dispersions in saline. The rats all received an experimental diet, *ad libitum* (they ate freely as much as they wanted), containing 1.27 umol (0.31 mg) biotin but without supplemental lipoic acid. It should be noted the low dose in rats corresponds to 62.3 mg lipoic acid/day and the high dose 225.4 mg lipoic acid/day with 34.3
mg biotin for a 70 kg human but without consideration of the PK differences between the routes of administration (humans eat it and rats got injected in the gut) or the carrier. In general, i.p. injections of the salts of lipoic acid produce faster and higher plasma and tissue concentrations than per oral (p.o) doses and the elimination ½ live (t ½) is longer.

Zempleni et al Results and Conclusions

Briefly, Zempleni et al found the activities of pyruvate carboxylase and B-methyl-crotonyl-CoA carboxylase (2 of 4 biotin dependent enzymes) in livers from the high dose, i.p. lipoic acid-treated rats were less than in controls (the activity was 64-72% of control values). They concluded similar effects would likely be found after chronic oral (p.o.) administration of lipoic acid in humans with inborn lack of B-carboxylase activity who could suffer “deleterious effects.” (see 3B below). In the high dose group lipoic acid: biotin was administered in a molar ratio of 7.8:1 and co-administration of biotin prevented the reduction in carboxylase activities.

Critique of Zempleni et al

3- The study being utilized to justify the “lipoic acid-biotin mandate” was done by two respected and frequently published biotin researchers (Zempleni & Mock; the third, Trusty is not as well known). The authors relied on a unique dosage form in a non-validated rat model (i.p. injection of test articles dispersed in PC/saline) as well as a suspected reporting error in lipoic acid PK data (incorrect elimination ½ live or t ½ [explained below]), utilized “heroic” doses of biotin and thus made conclusions that have been erroneously extrapolated to normal human lipoic acid consumers by scientists, doctors and nutritional supplement companies. There is no evidence the results of this study indicate lipoic acid poses any adverse health implications for humans with or without carboxylase deficiencies. It is also important to point out that an earlier study by Weiner & Wolf whose model more correctly mirrored lipoic acid PK in humans found contradictory results; i.e. lipoic acid DID NOT cause a reduction in the activity of any of the carboxylase enzymes in normal or even in rats starved of biotin.

A) Non-validated Dosage Forms: Most users of lipoic acid have heard it is both fat and water soluble and this is one of its unique properties over other so-called “antioxidants” they are usually quite surprised to discover this is only true after it has been diluted subsequent to absorption from the gut into the body. Approximately 0.1 % of solid, powdered lipoic acid is water soluble (wt/wt; 0.1 g dissolves in 99.9 g water). How then do researchers inject it? Generally, lipoic acid is converted into a completely water soluble salt by mixing it with an alkaline material such as sodium or potassium hydroxide (or other bases) and then adjusting it immediately before use to a physiological pH (~7.4). The study by Zempleni et al instead used dispersions of lipoic acid and biotin in PC/saline. I was unable to find any other studies (animal or PK) using this method of dispersing the test compounds and so it is impossible to state what effect this would have on subsequent plasma or tissue levels. Although the authors did not give details on how the dispersion was prepared this is usually done with a homogenizer in order to mix oil and water layers. In general this is how liposomes and nanospheres are made which can significantly increase plasma and tissue levels above those of powdered or p.o. lipoic acid. Therefore the results of this study may have less to do with the dose of lipoic acid used and more to do with how it was dispersed.

B) Non-validated Route of Administration: Intraperitoneal (i.p.) administration of lipoic acid and supplemental biotin was used in a rat model and the results were extrapolated to humans who consume lipoic acid and biotin orally without consideration of the pharmacokinetic differences between the two species or the routes of administration.

While the i.p. dose is frequently used in rat studies due to ease of administration, there is scant plasma PK data or resulting tissue concentrations reported in the literature for this route (and none for PC/saline dispersion). The i.p. dose involves injection of the test substances into the peritoneal cavity which causes saturation of the tissues contained in the cavity, followed by absorption as tissues are perfused with blood. Therefore plasma levels of the test substance after i.p. injection do not correlate well to tissue levels of the same compound consumed orally. In other words, tissue concentrations could be much higher than what is reflected in the plasma and this could cause the reduction in the activities of the biotin dependent enzymes.

Zempleni et al state that the pharmacokinetics of lipoic acid was “independent of the route of administration” which was based on the findings of two earlier studies which were apparently conflated.
“The great bioavailability of i.p. and orally administered lipoic acid has been reported previously in studies on the metabolism and the pharmacokinetics of radio-labeled lipoate (Peter and Borbe 1995, Spence and McCormick 1976).”

The above quote and the way it is referenced is misleading since it implies both papers confirmed the results of the other by testing the equality of PK measurements by each of the three routes of administration. Spence and McCormick tested only labeled i.p. and p.o. doses of lipoic acid in rats but did not determine the PK profiles. They only analyzed the radio-labeled content of plasma and tissues at two later time points (4 hr and 24 hr) which precludes the possibility of determining the concentration versus time curve. Peter & Borbe tested the p.o. and i.v. doses but not the i.p. dose used by Zempleni et al. Therefore even if Peter & Borbe had not made a possible reporting error (discussed below) reliance on their PK data by Zempleni et al is tenuous.

Krone compared the PK directly of i.p., intravenous (i.v.) and p.o. doses of 20 mg/kg R-lipoic acid (as a salt) in rats. She found p.o. and i.v. doses had similar t ½ values (despite considerable differences between the maximum plasma concentrations [C_max]) but the i.p. dose did NOT. In fact the absorption and elimination ½ lives overlapped with the i.p. dose such that rather than being roughly 0.5 – 0.75 hrs the i.p. dose was 5.58 hrs. Note this is still 10 fold lower than the results reported by Peter & Borbe and relied upon by Zempleni et al to reach their conclusions. Krone concluded rat PK (particularly the i.p. dose) was not a reliable model readily extrapolated to humans.

I suggest the difference in tissue concentrations between the low and high doses of lipoic acid rather than the “allegedly” long t ½ of lipoic acid accounts for the findings of this study. Zempleni et al speculate the chronic administration of the high dose of lipoic acid without supplemental biotin:

1-competitively inhibits biotin uptake & transport or
2-displaces biotin from the holocarboxylase synthetase enzyme or
3-displaces biotin from the carboxylases

The most important questions directly related to the study findings are:

1-Why did the high dose but not the low dose cause a reduction in the biotin dependent enzymes?
2-How did supplemental i.p. doses of biotin compensate for the high tissue concentrations of lipoic acid resulting from i.p. dosing?

There are no published reports of plasma PK studies where lipoic acid and biotin are administered simultaneously. The mechanism for the biotin sparing effect is likely complicated and was not determined in this study. I assume the addition of 85.6 ug/day i.p. above the amount obtained in the daily diet (average daily p.o. intake=26 nmol/day=6.35 ug/day) causes either sufficient tissue saturation or high tissue levels for a long enough period of time that it successfully competes with lipoic acid since as we have shown all lipoic acid PK studies indicate a short rather than long t ½. Contrary to this suggestion, high doses of biotin are known to down-regulate (reduce the number of enzymes encoded by DNA and translated into protein) the holocarboxylase, whereas low doses induce up-regulation (increase the number of enzymes encoded by DNA and translated into protein) in order to effectively bind miniscule amounts of available biotin. Possibly, simultaneous presence of both prevents normal down regulation.

C) Reliance on Possibly Erroneous Pharmacokinetic Data: Conclusions about the possible health implications for humans with carboxylase deficiencies were based on a probable reporting error in the t ½ of lipoic acid. The potentially adverse effect of lipoic acid on the activities of the biotin-dependent carboxylase enzymes was based on the necessity of chronic administration of lipoic acid believed by Zempleni et al to be in the circulation and tissues over one-two ½ lives (the time it takes for ½ the carboxylase enzymes to turn over) of the carboxylase enzymes by patients with inborn lack of B-carboxylase activity. This conclusion was based on a suspected reporting error in the pharmacokinetic (PK) data for p.o. and i.v. doses and the belief that the PK for the i.p. was essentially the same.
Zempleni et al relied on PK data provided by Peter & Borbe who reported the t ½ for p.o. lipoic acid of 51.9 hrs and that of i.v. lipoic acid 60.6 hrs.. This caused Zempleni et al to conclude that chronic administration and the long t ½ for lipoic acid would overlap the turnover times for the carboxylase enzymes and prevent new biotin from binding to the intermediate holocarboxylase synthetase enzyme (the enzyme responsible for transferring free biotin to the apocarboxylase enzymes) or from biotin from becoming incorporated into the apocarboxylase enzymes (the carboxylase enzymes without the biotin substrates) due to possible competition with lipoic acid due to structural similarities between the two molecules.

We reported the average PK values from all the published human PK studies and later Shay et al published the complete PK table of published studies demonstrating human t ½ values 100 times lower than those reported by Peter and Borbe. Therefore p.o. lipoic acid would NOT be in the circulation or tissues long enough or at sufficient concentrations to overlap the t ½ of the carboxylase enzymes. Although the German journal, Arzneimittelforschung (Drug Research) did not publish a correction for the values reported by Peter and Borbe, I suspect the correct t ½ values, based on ALL other animal and human PK studies should be 0.519 hrs for the p.o. dose and 0.606 hrs for the i.v. dose of lipoic acid.

Alternatively, Zempleni et al might have been correct about the long t ½ which could be unique to their dosage form and route of administration but due to the reasons cited above would not apply to orally consumed lipoic acid by humans.

In contrast to the findings of Zempleni et al the in vitro study and experimental conditions used by Wiener & Wolf may be more relevant than those used by Zempleni et al since the short incubation times used in their study (3-24 hrs) more closely mirrors the plasma and urinary elimination kinetics of lipoic acid in humans. All plasma and urinary absorption and elimination kinetic studies have shown the bulk of administered lipoic acid is cleared from plasma within 3-8 hours, time points corresponding to the maximum concentration of urinary metabolites. Interestingly, Peter and Borbe reported 78 and 84% of labeled lipoic acid was recovered in the 24 hr urine from the p.o. and i.v. doses. This appears to be irreconcilable with the reported plasma t ½ of 51.9 hrs and 60.6 hrs.

Tissue levels return to baseline within 24 hours. Takenouchi et al demonstrated 91-99% of the radioactivity was recovered in the 24 hr urine of humans after administration of 1 mg labeled 35-S (hot) lipoic acid (the sulfur atoms at the 6 and 8 positions have been replaced by radioactive sulfur). This study provides further evidence the t ½ reported by Peter and Borbe and relied upon by Zempleni et al is incorrect and that lipoic acid is not in the body long enough to interfere with the activity of the carboxylases.

- Zempleni et al used racemic (+/-) lipoic acid not R-(+)- lipoic acid so it is NOT technically correct to assume R-lipoic acid would behave similarly since PK, tissue localization and concentration differences between the 3 forms of lipoic acid are known.

Zempleni et al used rac-lipoic acid so it is unknown if the single enantiomers of lipoic acid (R-lipoic and S-lipoic acid) would cause similar reductions in the carboxylase activities even under identical experimental conditions. Even if such a reduction occurred the relevance to humans is not clear. The justification of using the racemate in the present study by claiming d and l-lipoic acid (d-LA= (+)-LA=RLA and l-LA= (-)-LA=SLA) were similarly catabolized by microorganisms is misplaced, since rats and humans are NOT microorganisms and catabolism of lipoic acid was not shown in this study to have any influence on the results or conclusions. Evidence demonstrates the single enantiomers and the racemic mixture (R/S-lipoic acid) have different PK profiles, stereoselective differences in absorption, tissue localization and metabolism in both intact humans and in rat liver.

In the discussion section of the lipoic acid-biotin study as well as in a subsequent review article Zempleni backed off of the strong warnings concerning the possible relevance of the study to human lipoic acid users with carboxylase deficiencies.

"However the pathological implications of such a decrease are not clear. Individuals who are heterozygous for pyruvate carboxylase deficiency, B-methylcrotonyl-CoA carboxylase deficiency and propionyl CoA carboxylase deficiency have carboxylase activities approximately 50% of normal and yet are characteristically asymptomatic."

This has been overlooked by other lipoic acid researchers and ALL of the companies advocating the "lipoic
acid-biotin mandate” relying on Zempleni et al as definitive. So the question remains, why the strong language if humans with inborn carboxylase deficiencies have lower enzyme activities than rats administered lipoic acid and yet are asymptomatic?

Interestingly, Zempleni et al issued warnings about human lipoic acid use but without going so far as to recommend supplemental biotin to compensate even though their study showed supplemental biotin (in a molar ratio of 7.8 to 1; lipoic acid to biotin) administered i.p. with the high dose of i.p. lipoic acid maintained the normal activities of the carboxylase enzymes.

I suspect this omission was due to the amount of biotin needed (based on dose/body weight) far exceeds what is considered to be the daily adequate intake (AI) level. Based on a dose equivalency [and ignoring the differences in the PK values for the i.p. and p.o. dose] a 70 kg human lipoic acid user consuming 225.3 mg/lipoic acid/day (equivalent to the rat high dose) would need to consume 34.3 mg biotin/day or 1141 times greater than the amount considered to be the adequate intake (AI=30 ug/day). A therapeutic dose of 600 mg lipoic acid would require ~91.3 mg biotin. I suspect Zempleni et al were NOT comfortable making a recommendation for such a “mega-dose”. As a point of reference, the average human diet provides 35-70 ug/day.10

I suggest the results of this study were due to unique problems associated with the study design or to PK differences between PC/saline and salt dosage forms of lipoic acid as well as the differences between the i.p. route of administration used in rats and the p.o. does used by humans.

According to Zempleni et al:

“We administered lipoic acid i.p. but these results likely will be found with chronic oral administration.” As discussed above this conclusion is not likely correct.

Conclusions: The existing evidence and critical analysis of Zempleni et al indicates it is NOT correct to assume the results of this study can be extrapolated to humans with or without carboxylase deficiencies or that the three forms of lipoic acid or different dosage forms would produce equivalent results in either rats or humans. The extrapolation of the results of this study to the lipoic acid using public is unwarranted. If supplemental biotin is desired by lipoic acid users it is likely better to consume biotin at least three hours after lipoic acid to insure both nutrients are maximally absorbed.

The “lipoic acid-biotin” mandate provides a clear example that even peer reviewed literature should be used as a guide to further research and should not be considered as absolute, unless the study is comprehensive and/or critically reviewed and replicated so that final determinations of its relevance can be made. It also demonstrates that long held theories based on scientific errors may be at a later time corrected. This study has been in the public domain for 12 years and accepted as a matter of fact and further extrapolated to healthy lipoic acid users by scientists, doctors and nutritional companies alike. Let’s see how Zempleni et al and the J of Nutrition respond to my critique.

Even scientists are guilty of over extrapolating the results of this study. At a recent scientific conference a well known professor said to me “Did you know lipoic acid depletes the body of biotin”? Since biotin deficiencies are relatively rare and adequate biotin can be derived from the diet or from gut microbes and is found in any B-complex formula, I don't see any advantage of taking it in the same formula or at the same time as lipoic acid. Due to PK differences between p.o. lipoic acid and p.o. biotin, staggering the times of consumption should insure there is always sufficient biotin available to the apocarboxylases without competition or displacement by lipoic acid. Rather than relying on theories the relevance of the Zempleni et al study would be relatively simple to determine by testing whether or not normal, healthy lipoic acid users or those with carboxylase deficiencies are biotin deficient by testing the urinary output of biotin and increases in the 3-hydroxyisovaleric acid, an established marker for deficiency of B-methyl-crotonyl-CoA carboxylase activity and one of the enzymes affected in this study.10

An underappreciated study indicated lipoic acid can stimulate the biosynthesis of biotin.12 Humans have lost the ability to synthesize biotin which is why it is classified as a vitamin but our gut flora can produce it possibly in response to lipoic acid consumption. So rather than depleting the biotin dependent enzymes, lipoic acid might stimulate our gut flora to produce it thus preventing any deficiencies. This is a fascinating
proposal with health implications and should be studied further.

A question I’d like to ask the vendors of R-lipoic acid advocating concurrent use of biotin is why they would make such a fuss about using the natural R-(+)-form of lipoic which is expensive and has a very low bioavailability compared to the cheap, common form of lipoic acid and then add the cheap d, l-unnatural form of biotin to it? Additionally the bioavailability of the free-acid form of R-lipoic acid (the form found in most of the supplements by companies advocating the lipoic acid-biotin mandate) is so low there is no danger of it inhibiting biotin or anything else, except consumer cash flow.


13- Carlson DA, Smith AR, Fischer SJ, Young KL. A comparison of the pharmacokinetic profiles of lipoate enantiomers with the racemic mixture in healthy human subjects indicates possible stereoselective gut transport.

View the poster from OCC Santa Barbara, CA March 12-15, 2008