Biotin Biotransformation to Bisnorbiotin Is Accelerated by Several Peroxisome Proliferators and Steroid Hormones in Rats

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ABSTRACT  Bisnorbiotin and biotin sulfoxide are the major catabolites of biotin for humans, swine, and rats. Increased urinary excretion of bisnorbiotin, biotin sulfoxide, or both have been observed during pregnancy and in patients treated with certain anticonvulsants. We sought more insight into the process of biotin catabolism in mammals by exposing rats in vivo to compounds known to induce classes of enzymes that were candidates to catalyze the biotransformations. Rats were treated with the anticonvulsants phenytoin, phenobarbital, and carbamazepine, the steroid hormones dexamethasone and dehydroepiandrosterone, and the peroxisome proliferators clofibrate and dl(2-ethylhexyl)phthalate. [14C]Biotin was injected intraperitoneally at physiologic doses in treated rats and control rats; HPLC and radiometric flow detection were used to specifically identify and quantify [14C]biotin and its metabolites in urine. Treatment effects were assessed by the change in the urinary excretion of [14C]bisnorbiotin and [14C]biotin sulfoxide in response to administration of [14C]biotin. No significant changes resulted from treatment with any of the anticonvulsants. With the steroid hormones and the peroxisome proliferators, [14C]bisnorbiotin excretion increased significantly. These results indicate that biotin is converted into bisnorbiotin in the liver and that this conversion likely occurs in peroxisomes or mitochondria or both via \( \beta \)-oxidative cleavage, and, in contrast to responses in humans, the enzymes responsible for the formation of biotin sulfoxide in rats are not induced by the anticonvulsants examined here.  J. Nutr. 127: 2212–2216, 1997.

KEY WORDS:  anticonvulsant  •  biotin  •  bisnorbiotin  •  peroxisome proliferator  •  rats

The water-soluble vitamin biotin acts as a coenzyme for four carboxylases in mammals. These carboxylases catalyze component steps of fatty acid biosynthesis, gluconeogenesis, amino acid metabolism, and propionate metabolism. The major catabolites of biotin for humans, swine, and rats are bisnorbiotin (BNB) and biotin sulfoxide (BSO).

Observations in two groups of patients have stimulated our interests in the catabolism of biotin. Studies from our laboratory have indicated that biotransformation of biotin to BNB and BSO is increased by long-term treatments with anticonvulsants (Mock and Dyken 1997). These studies and those from the laboratory of Krause and co-workers (1982a, 1984 and 1988) suggest that anticonvulsant therapy may also result in reduced biotin status in some individuals. We have observed significantly increased biotin biotransformation to BNB in normal pregnancy. Some of our observations suggest that about half of the women studied became marginally biotin deficient late in pregnancy and that those pregnant women with increased excretion of BNB were more likely to fall into the deficient group (Mock, D. M. et al. 1997a).

Bisnorbiotin differs from biotin by a two-carbon reduction of the side chain and oxidation of the sulfur of biotin. Early in pregnancy, the presence of a \( \beta \)-oxidation pathway for the side chain and oxidation of the sulfur of biotin in microbes was demonstrated by Ogata and co-workers (Yang et al. 1968) and by McCormick and co-workers using radiolabeled biotin as the sole carbon source (Iwashara et al. 1969, Kazarino et al. 1972). These oxidations were also found to occur in rats (Lee et al. 1972). Hence, the assumption that BNB is formed in mammals by enzymatic \( \beta \)-oxidation is reasonable. However, direct proof of the enzymatic nature of the biotransformation in mammals is minimal, and the organ site(s), the subcellular site(s), and the enzyme(s) responsible remain unknown. For example, is this biotin \( \beta \)-oxidation simply the result of catalysis by one or more of the mitochondrial or peroxisomal fatty acid \( \beta \)-oxidation enzymes? If so, the rate of biotin biotransformation would not down-regulate importantly early in the course of progressive biotin deficiency. However, our studies in which biotin deficiency was induced experimentally by egg white feeding provide evidence that the urinary excretion of BNB does decrease early in biotin deficiency (Mock, N. I. et al. 1997).

For these reasons, we sought more insight into the process of biotin catabolism in mammals by exposing rats in vivo to compounds known to induce classes of enzymes that are candidates to catalyze the biotin biotransformation. The effect
on biotin biotransformation was assessed by measuring changes in the urinary metabolite profile.

MATERIALS AND METHODS

Chemicals and reagents. Clofibrate, phenobarbital, and dehydroyiandrosterone (DHEA) were purchased from Sigma Chemical (St. Louis, MO). Phenytoin, carbamazepine, and d(2-ethylhexyl)phthalate (DEHP) were purchased from Aldrich Chemical (St. Louis, MO). Dexamethasone sodium phosphate was obtained in injectable form at a concentration of 100 mmol/L (American Reagent Laboratory, Shirley, NY). D-(14C-carbonyl)biotin with a specific radioactivity of 2 GBq/mmol was obtained from Amersham (Arlington Heights, IL). D-[8,9-3H(N)]Biotin with a specific radioactivity of 1.7 TBq/mmol was purchased from DuPont NEN (Boston, MA). The synthesis and characterization of radiolabeled biotin analogs have been described elsewhere (Wang et al. 1996).

Animals and diets. Male Sprague-Dawley rats (Harlan, Madison, WI) were provided a biotin-sufficient diet (PROLAB R-M-H 3000, Agway, Syracuse, NY), for at least 1 wk to stabilize their biotin nutritional status. The diet contains 1.6 nmol biotin/g. The same diet was provided during the experimental period. The proximate analysis of the diet is 22.5% protein, 5.5% fat, 4.5% fiber, 5.5% ash, 10% moisture and 52% nitrogen-free extract. Animal protocols were approved by the University of Arkansas for Medical Sciences Animal Care and Use Committee.

Response to anticonvulsants, steroid hormones and peroxisome proliferators. The treatment, doses, duration, and route of administration for each compound tested are listed in Table 1. A single control group was used for the comparison to all the treated groups. After treatment, rats were injected with a single physiologic dose of [14C]biotin (57 pmol/g body wt). The accelerated biotin β-oxidation was judged by comparing the urinary biotin metabolite profile in treated groups to that of the control group.

Sample collection and the determination of total urinary radioactivity. After the administration of [14C]biotin, rats were housed in individual metabolic cages (Nalge, Rochester, NY) for urine sample collection. These metabolic cages discretely separated urine from feces. The radioactivity excreted in feces (<5%) accounted for only a very small proportion of administrated [14C]biotin, and therefore subsequent studies focused only on urinary metabolites (Wang et al. 1996). Radioactivity in urine was determined in triplicate by liquid scintillation counting using Ultima Gold (Packard, Meriden, CT) scintillation fluid and quench correction. Total urinary excretion of radioactivity was calculated from timed urine volumes and concentrations of radioactivity.

Biotin metabolite profile. Radiolabeled biotin metabolites in urine were separated by HPLC and quantified by radiometric flow detection as described previously (Mock et al. 1993). Briefly, radioactivity in the HPLC eluate was quantified using a radiometric flow detector (Radiomatic Flo-One/Beta Radiochromatography Analyzer, Series A-500, Packard Instrument). The HPLC flow conditions and scintillation fluid were as described previously (Wang et al. 1996). Radiolabeled biotin, BNB, and BSO were identified by their retention times compared with [3H]biotin, [14C]BNB, and [3H]BSO synthesized as described previously (Mock et al. 1992, Wang et al. 1996). The d- and l- forms of BSO stereoisomers are not specifically resolved by this HPLC system. Therefore, in this study, BSO refers to the sum of these two isomers (Mock, D. M. et al. 1997b, Wang et al. 1996).

Statistical methods. Data are presented as means ± SD. Differences in the 24-h cumulative urinary radioactivity among all groups were tested by one-way ANOVA. Differences in the means of the urinary biotin or metabolites between each treatment and the control groups were tested for significance using ANOVA with Dunnett’s post-hoc procedure (Zar 1974). Statistical analyses were performed with a standard statistical package (StatView, Abacus Concepts, Berkeley, CA). For all tests, significance was set at P < 0.05.

RESULTS

Urinary excretion of total radiolabeled biotin plus metabolites. Depicted in Figure 1 are the cumulative urinary radioactivity data for the various treatment groups and control group. The cumulative excretions at 72 h varied from 55 to 75% of the injected radioactivity, but a consistent trend to increase or decrease with treatment was not apparent. The curve shapes were similar in all groups; the major portion of administered radioactivity was excreted within 24 h of the injection. Analysis of variance of the cumulative excretion at 24 h revealed no significant differences.

Effect of treatment on urinary biotin metabolite profiles. Depicted in Figure 2 are the metabolite excretion data for BNB

### TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Dose (µmol/g body wt)</th>
<th>Duration</th>
<th>Administration route</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Phenytoin</td>
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<td>3</td>
<td>i.p.</td>
<td>Kim et al. 1993</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>3</td>
<td>0.3</td>
<td>3</td>
<td>i.p.</td>
<td>Corcos 1992</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>6</td>
<td>0.3</td>
<td>3</td>
<td>i.p.</td>
<td>Wu et al. 1989</td>
</tr>
<tr>
<td>Dehydroxypregnandrosterone</td>
<td>6</td>
<td>0.2</td>
<td>3</td>
<td>Diet</td>
<td>Morgan and Badawy 1992</td>
</tr>
<tr>
<td>Clofibrate, acute high dose</td>
<td>6</td>
<td>1.7</td>
<td>3</td>
<td>i.p.</td>
<td>Sundseth and Waxman 1992</td>
</tr>
<tr>
<td>Clofibrate, therapeutic dose</td>
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<td>0.24</td>
<td>14</td>
<td>Diet</td>
<td>Sallustio 1991</td>
</tr>
<tr>
<td>D-[2-ethylhexyl]phthalate</td>
<td>5</td>
<td>4.0</td>
<td>7</td>
<td>Diet</td>
<td>Osumi and Hashimoto 1978</td>
</tr>
</tbody>
</table>

1 The control group was used for the comparisons to all the treated groups. Data of the control group have been reported in the cited reference (Wang et al. 1996).

2 Intraperitoneal injection (i.p.) dissolved in <1 mL of 154 mmol/L NaCl solution.

3 Dissolved in 95% ethanol, applied to diet pellets, and oven-dried to achieve drug:diets weight ratio.

4 The dose was calculated from the average diet intake and the drug:diets weight ratio. This dose is similar to that used for treating hyperlipidemia in humans.
and co-workers (Yang et al. 1968) and by McCormick and co-workers (Iwahara et al. 1969, Kazarinoff et al. 1972). Moreover, the presence of bisnorbiotin methyl ketone in urine of human subjects (Zempleni et al. 1997b) provides additional support of the hypothesis that BNB is formed by an enzymatic \( \beta \)-oxidation, because this methyl ketone probably arises from a less stable \( \beta \)-keto acid intermediate in a fashion analogous to the enzymatic \( \beta \)-oxidation of fatty acids (Lee et al. 1972, Kazarinoff et al. 1972). Notwithstanding these pioneering observations, little is known about the primary organ sites, subcellular organelle sites, identities, characteristics, or regulation of the enzymes responsible for biotin catabolism to BNB.

This study provides evidence that the biotin biotransformation to BNB is inducible by certain peroxisome proliferators and steroid hormones. Of course, this conclusion is based in part on the assumption that urinary excretion of BNB reflects formation of BNB that is in turn directly related to the enzyme activity induced by each treatment. Separate studies from our laboratory detect very little biliary excretion of biotin or metabolites (Zempleni et al. 1997a), confirming that urine is the predominant route for excretion of biotin and its metabolites.

**DISCUSSION**

The inactive metabolite BNB differs from biotin by a two-carbon reduction in the length of the valeric acid side chain. This two-carbon reduction is a common motif for the enzymatic \( \beta \)-oxidation of saturated straight-chain fatty acids of various lengths. Thus, it would be reasonable to assume that the biotransformation of biotin to BNB is an enzyme-catalyzed process. Indeed, the presence of a \( \beta \)-oxidation pathway for the side chain of biotin was demonstrated in microbes by Ogata (Fig. 2A) and BSO (Fig. 2B) after treatment with anticonvulsants (phenytoin, phenobarbital, and carbamazepine), steroid hormones (dexamethasone and DHEA), or peroxisome proliferators (clofibrate and DEHP).

Neither the absolute excretion (data not shown) nor the relative proportion of BNB changed significantly in response to the treatment of rats with the three anticonvulsants. Likewise, neither the absolute nor relative excretion of BSO changed in response to anticonvulsants.

In response to treatment with the two steroid hormones or the two peroxisome proliferators, BNB excretion in the treated groups was higher than that in the control group. Biotin sulfoxide excretion was not significantly different between either the hormone-treated groups or the peroxisome proliferator–treated groups and the control group.

Our initial study with clofibrate used an acute high dose treatment regimen (CFa) commonly used in enzyme induction studies in rats (Grafnetter et al. 1971, Sundseth and Waxman 1992). We wondered whether longer treatment with doses similar to those used for treatment of hyperlipidemia (CFc) would also produce significant changes in BNB excretion. The increased excretion of BNB was similar to the increase seen with the acute dose; again no significant differences were seen in BSO excretion.

![DISCUSSION](https://academic.oup.com/jn/article-abstract/127/11/2212/4728652)
Both the steroid hormones and the peroxisome proliferators studied here belong to the steroid hormone receptor superfamily of ligand-activated transcription factors and exert their effects through a receptor-mediated mechanism involving peroxisome proliferator-activated receptors (Anderson et al. 1996, Green 1995, Issmann and Green 1990). In general and for the particular peroxisome proliferators studied here, treatment increases both the number and volume of peroxisomes in rodent liver and causes a marked induction of lipid-metabolizing enzymes, including cytochrome P450-dependent fatty acid \( \omega \)-hydroxylases (Hawkins et al. 1987), the enzymes of the peroxisomal fatty acid \( \beta \)-oxidation (Lazarow and De Duve 1976), and some mitochondrial enzymes (Hawkins et al. 1987) including mitochondrial fatty acid \( \beta \)-oxidases, where examined. As indicated in Table 2, the induction of fatty acid \( \beta \)-oxidation by the steroid hormones and peroxisome proliferators used here cannot be categorized as organelle specific. Indeed, the similarity in \( \beta \)-oxidases of fatty acid in peroxisomes and mitochondria might reflect some degree of coordinate regulation. Thus we cannot reasonably exclude either peroxisomes or mitochondria as the source of the induced enzyme activity. Likewise, the known hepatic effects of peroxisome proliferators do not exclude the possibility that other organs besides liver participate in biotin \( \beta \)-oxidation and are responding to the \( \beta \)-oxidase inducers.

The fatty acid \( \beta \)-oxidation in peroxisomes is similar to the four-step mitochondrial fatty acid \( \beta \)-oxidation; however, the initial and rate-limiting dehydrogenation step is catalyzed by an acyl-CoA dehydrogenase (Reddy and Mannaeerts 1994) rather than by acyl-CoA dehydrogenase. Increased peroxisomal \( \beta \)-oxidation is often used as a biochemical indicator of the peroxisome proliferation effect and as a rapid and sensitive method to identify hypolipidemic drugs (Anderson et al. 1996, Lazarow et al. 1982). We speculate that these steroids and peroxisome proliferators induce biotin biotransformation enzymes in a manner similar to induction of fatty acid \( \beta \)-oxidation enzymes.

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Mitochondrial Acyl-CoA dehydrogenase</th>
<th>Peroxisomal Acyl-CoA oxidase</th>
<th>Cytochrome P450 4A</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid hormone</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td>Yes</td>
<td>?</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypolipidemic agent</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Plasticizer</td>
<td>Di(2-ethylhexyl)phthalate</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

\(^1\) Known peroxisome proliferator (PP).

Labeled \([^{14}C]\)biotin. Their work examined the inhibitory effects caused by various fatty acids added to the medium on biotin oxidation as well as inhibitory effects of biotin and biotin analogs on butyrate oxidation. They concluded that separate enzyme systems were involved in biotin and fatty acid oxidation. However, these inhibitory interactions could have occurred 1) at the level of transport into the cell, 2) via effects on the energy supply, 3) at the level of conversion of biotin to biotinyl-CoA, 4) at the level of the transport into the mitochondria or peroxisomes or 5) at the level of competition for the substrate binding site on one or more of the fatty acid or biotin \( \beta \)-oxidation systems. Thus it is not reasonable to conclude from the study of Baxter and Quastel (1953) (or from our current study) whether there are discrete systems for biotin \( \beta \)-oxidation and fatty acid \( \beta \)-oxidation. However, it is clear from our studies of patients treated with anticonvulsants, from studies in which biotin deficiency was induced experimentally, and from the biotransformation studies presented here that biotin catabolism can be influenced by biotin status and by factors such as medication and drug therapy that are not directly related to biotin status.

Long-term treatment with anticonvulsants has been reported to increase biotin catabolism (Mock and Dyken 1997) to BNB and BSO and to lead to marginal biotin deficiency (Krause et al. 1982a, 1982b and 1984, Mock and Dyken 1997). In the current study, none of the anticonvulsants accelerated the formation of BNB or BSO. This failure to reproduce the increased BSO excretion seen in humans may relate to species differences in inducibility of the various P450 isoenzymes and their related substrate specificities. Other candidates for the sulfoxidation reaction include the flavin monooxygenase. Likewise, the failure to reproduce anticonvulsant-accelerated BNB production may be species related. Whether BNB production is accelerated in humans by steroid hormones or peroxisome proliferators is not known.

In conclusion, the fact that biotin \( \beta \)-oxidation can be increased by steroid hormones and peroxisome proliferators that also induce fatty acid \( \beta \)-oxidation suggests that biotin is catabolized by an enzymatic pathway that is similar to that of fatty acids and that pathway may include one or more of the fatty acid oxidases. Failure of the three anti-convulsant drugs to alter biotin metabolism suggests that the microsomal mixed-function enzymes that are inducible by these drugs in rats are not rate-limiting in biotin sulfoxidation or \( \beta \)-oxidation in rats.
LITERATURE CITED


