NADPH-dependent Reductive Metabolism of Cis-5 Unsaturated Fatty Acids

A REVISED PATHWAY FOR THE $\beta$-OXIDATION OF OLEIC ACID*

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Kou-Yi TserngT and Shiow-Jen Jin
From the Medical Research Service, VA Medical Center and the Departments of Pharmacology and Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

Cis-5 double bond in a fatty acid or when encountered through the $\beta$-oxidation of an odd-numbered double-bond unsaturated fatty acid presents as a metabolic block to the further $\beta$-oxidation. Cis-5-fatty acyl-CoA cannot be $\beta$-oxidized to cis-3-enoyl-CoA as suggested by the conventional pathway. Instead, this metabolic block can only be removed through an NADPH-dependent reduction of 5-enoyl-CoA, possibly mediated by a 5-enoyl-CoA reductase. In the case of oleic acid, two cycles of $\beta$-oxidation yield cis-5-tetradecenoyl-CoA. This intermediate is then reduced to tetradecenoyl-CoA, which is metabolized further via normal $\beta$-oxidation cycles. The conventional pathway through cis-3-dodecenoyl-CoA does not operate in rat liver.

Stoffel and Caesar (1) proposed a metabolic pathway for odd-numbered double-bond unsaturated fatty acids, such as oleate, through a stepwise $\beta$-oxidation to a cis-3-enoyl-CoA intermediate. The conversion of cis-3-enoyl-CoA to trans-2-enoyl-CoA is then mediated by $\Delta^2$-cis-$\Delta^2$-trans-enoyl-CoA isomerase. This is followed by the normal $\beta$-oxidation sequence from trans-2-enoyl-CoA, which leads to complete oxidation of the unsaturated fatty acid to acetyl-CoA. The existence of a $\Delta^2$-cis-$\Delta^2$-trans-enoyl-CoA isomerase in rat liver mitochondria and peroxisomes has been demonstrated (2-4). However, the conversion of cis-5-enoyl-CoA to cis-3-enoyl-CoA by one cycle of $\beta$-oxidation has never been documented. Experimental evidence indicates that a cis-5 double bond in fatty acids might present as a metabolic obstacle to further $\beta$-oxidation (5). Fatty acids with a terminal cis-5 double bond, such as arachidonic and eicosapentaenoic acids, are known to have unusually low metabolic rates in rat liver mitochondria and peroxisomes (6-9).

We have recently identified the unsaturated decenoic and dodecenoic acids in urine as mainly cis-5 and cis-4 structures (5, 10). This implies that the metabolism of a cis-5 double bond could be at rate limiting as is already known for the NADPH-dependent pathway of the metabolism of a cis-4 double bond (11). Furthermore, metabolic studies performed in vitro using rat liver homogenate also failed to demonstrate the metabolic conversion of a cis-5-dicarboxylic acid to a cis-3-dicarboxylic acid by one cycle of $\beta$-oxidation (5). Instead, these cis-5-dicarboxylic acids are metabolized preferentially from the $\omega$-end carboxyl, and the metabolic conversion is stopped when another cis-5 structure is encountered. No cis-3-dicarboxylic acid was produced in these experiments. However, saturated dicarboxylic acids of lower carbon chain were produced in significant amounts. These data suggest that a cis-5 structure presents a metabolic block; yet this block can be removed by direct reduction instead of further metabolism to a cis-3 double bond followed by isomerization. The following experiments present evidence for this reduction pathway.

**MATERIALS AND METHODS**

**Chemicals**—Cis-3-decenoic, cis-4-decenoic, cis-5-decenoic, cis-5-dodecenoic, cis-9-dodecenoic, and cis-5-tetradecenoic acids used as substrates were synthesized in this laboratory as described before (5). These preparations contain between 1 and 2% saturated fatty acids as determined by gas chromatography (5). 3-Hydroxydecenoic, 3-hydroxyoctanoic, 3-hydroxydodecanoic, and 3-hydroxypentadecanoic acids were prepared as reported before (12). Trans-3-decenoic acid was obtained from the isomerization of cis-3-decenoic acid (5). cis-7-Dodecenoic acid was prepared from the chromic acid oxidation of cis-7-dodecene-1-ol (Sigma). The structure was confirmed by electron impact (70 eV) mass spectrometry of trimethylsilyl ester. This product contains less than 1% dodecanoic acid. Other chemicals (decanoic, dodecanoic, tetradecanoic, and octanoic acids) were purchased from Aldrich. Co-factors (NADPH, NAD, ATP, and CoA) were supplied by Sigma. Oleic acid, oleyl-CoA, and stearoyl-CoA were purchased from Sigma.

**Isolation of the 10,000 x g Pellet from Rat Liver Homogenate**—Liver from nonfasting Holtzman rats was homogenized in 0.1 M phosphate buffer (pH 7.4). This 20% (w/v) homogenate was first centrifuged at 400 x g to remove the debris. The supernatant was then centrifuged at 10,000 x g to remove microsomes and soluble fraction. The pellet obtained was washed with phosphate buffer once. This preparation was suspended in phosphate buffer to a final protein concentration of 30-50 mg/ml; it was used fresh or kept frozen at -20 °C.

**Incubation Studies with the 10,000 x g Pellet**—A mixture of the suspended 10,000 x g pellet (100 μl), 3-5 mg of protein, ATP (10 μmol), CoA (5 μmol), and MgCl₂ (10 μmol) was incubated with 0.5 μmol of substrate in 3 ml of 0.1 M phosphate buffer (pH 7.4) at 37 °C in a Dubnoff shaking incubator from 10 to 120 min. The reaction was started by the addition of substrate and terminated by quick freezing in an acetone/dry ice mixture. In other experiments with additional co-factors NADPH (0.1 μmol), NAD (1 μmol), or both were added at the beginning of the experiment.

**Analysis of Metabolite in the Reaction Mixture**—An aliquot (1 ml) of the incubation mixture was mixed with pentane-decanic acid as the internal standard. After 90 °C for 30 min under alkaline conditions with the addition of 60 μl of 3 N KOH the hydrolyzate was acidified and extracted with a solvent mixture of ethyl acetate and diethyl ether, 1:1. The metabolites were analyzed as trimethylsilyl derivatives in a dual capillary column gas chromatograph equipped with a nonpolar SPB-1 (methyl silicone) column and an intermediate polarity SPB-35 (35% phenyl silicone: 65% methyl silicone) column (Supelco, Bellefonte, PA). Positive compound identification was obtained using a Hewlett-Packard 5890B gas chromatograph-mass spec-
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Unsaturated Cis-3-, Cis-4-, and Cis-5-fatty Acids—Under the same conditions, the metabolic disposition of cis-3-, cis-4-, and cis-5-decanoic acids was significantly slower than their saturated counterparts when the metabolic rates were assessed by the disappearance of the starting substrates. The relative rates to decanoic acid were 52% for cis-3-decanoic, 10% for cis-4-decanoic, 25% for cis-5-decanoic, 33% for cis-5-dodecanoic, and 44% for cis-5-tetradecanoic acids. As shown in Fig. 2B for cis-5-dodecanoic acid, the metabolism of cis-5-fatty acids is significantly slower than their saturated counterparts.

Unsaturated trans-2-, trans-3-, and cis-3-fatty acids—The metabolic origin of trans-3- and cis-3-fatty acids was confirmed by incubation of 3-hydroxydecanoic and 3-hydroxydodecanoic acids under identical conditions. An identical profile of trans-2-, trans-3-, and cis-3-fatty acids was observed. These unsaturated products were not detected when 3-hydroxy fatty acids were incubated under the same conditions but without the 10,000 × g pellet.

In view of the accumulation of 3-hydroxy fatty acids, the metabolic conversion under our experimental conditions was apparently limited by the availability of NAD, the co-factor required for the activity of 3-hydroxyacyl-CoA dehydrogenase. When NAD was added in some of the experiments using dodecanoic acid as substrate the metabolic rate of dodecanoic acid was not changed, but decanoic and octanoic acids became the major detectable metabolic intermediates. Because metabolic intermediates could be analyzed in this system of a rat liver preparation, the 10,000 × g pellet was used for the studies with unsaturated fatty acid substrates.

Metabolism of Unsaturated Cis-3-, Cis-4-, and Cis-5-fatty Acids—Under the same conditions, the metabolism of cis-3- and cis-4-fatty acids was significantly slower than their saturated counterparts when the metabolic rates were assessed by the disappearance of the starting substrates. The relative rates to dodecanoic acid were 52% for cis-3-decanoic, 10% for cis-4-decanoic, 25% for cis-5-decanoic, 33% for cis-5-dodecanoic, and 44% for cis-5-tetradecanoic acids. As shown in Fig. 2B for cis-5-dodecanoic acid, the metabolic conversion under our experimental conditions was apparently limited by the availability of NAD, the co-factor required for the activity of 3-hydroxyacyl-CoA dehydrogenase. When NAD was added in some of the experiments using dodecanoic acid as substrate the metabolic rate of dodecanoic acid was not changed, but decanoic and octanoic acids became the major detectable metabolic intermediates. Because metabolic intermediates could be analyzed in this system of a rat liver preparation, the 10,000 × g pellet was used for the studies with unsaturated fatty acid substrates.
Acids-The metabolite profile of cis-5-decenoic acid after a period of 60 min the composition of diunsaturated metabolite between carbons 3 and 4 of a 3-hydroxy fatty acid bistrimethylsilyl derivative on mass spectrum: cis-3-hydroxy-cis-5-decenoic acids. The metabolic origins of decadienoic acids based on the molecular ion, 3-hydroxy-cis-5-decadienoic, cis-3-cis-5-decadienoic, trans-3-cis-5-decadienoic, and 3-hydroxy-cis-5-decadienoic acids based on similar retention time on gas chromatography-mass spectrometry: 1, cis-5-decenoic; 2, cis-3-cis-5-decenoic; 3, trans-5-cis-5-decenoic; 4, trans-2-cis-5-decenoic; and 5, 3-hydroxy-cis-5-decenoic acids. B, the metabolism of cis-5-decenoic acid under the same conditions as A but with the additional NADPH. Under these conditions additional metabolites were detected. These metabolites are: 6, decanoic acid; 7, 3-hydroxydecenoic acid.

The metabolite profile of cis-5-decenoic acid after a 30-min incubation is shown in Fig. 4A. Peak 5 was assigned as the metabolite profile of cis-5-decenoic acid based on similar retention to 3-hydroxydecenoic acid and on mass spectrum: m/z (%) 315 (M+15%, 5%), 233 (100%). The mass fragment, m/z 233 CH(OH)(CH3)2CH2CO2Si(CH3)3, is derived from the cleavage between carbons 3 and 4 of a 3-hydroxy fatty acid bistrimethylsilyl derivative. This fragment is indicative of a 3-hydroxy group on the molecule. Peaks 2–4 are three isomeric decadienoic acids based on the molecular ion, m/z 240, and M+15 ion, m/z 225. Peak 4 has m/z 240 (20%), 225 (60%), 181 (15%), 169 (100%), and 155 (25%). Peaks 2 and 3 have identical mass spectra: m/z 240 (15%), 223 (20%), 196 (8%), and 169 (9%). Based on the retention time on gas chromatography peak 4 is most likely trans-2-cis-5-decadienoic acid since trans-2 isomers have the longest retentions among isomeric unsaturated fatty acids (18). Based on the analogous metabolism in decanoic acid (Fig. 3) and the relative retention times of these metabolites peak 2 is likely to be cis-3-cis-5-decadienoic acid, and peak 3 is trans-3-cis-5-decadienoic acid.

The metabolite profile after a 30-min incubation was cis-5-decenoic (63%), cis-3-cis-5-decadienoic (6%), trans-3-cis-5-decadienoic (6%), trans-2-cis-5-decadienoic (19%), and 3-hydroxy-cis-5-decadienoic (6%) acids. The metabolic origins of these metabolites are postulated in Fig. 5. After an incubation period of 60 min the composition of diunsaturated metabolite doubled, but the percentage of 3-hydroxy metabolite remained unchanged.

**Fig. 3.** Metabolism of decanoic acid in the rat liver 10,000 x g pellet with ATP, CoA, and MgCl2. Decanoyl-CoA (I) is metabolized rapidly to 3-hydroxydecanoyl-CoA (V) through the intermediate trans-2-decanoyl-CoA (II). The accumulated 3-hydroxy-decanoyl-CoA is then converted to cis-3-decanoyl-CoA (III) and trans-3-decanoyl-CoA (IV) either by direct enzymatic dehydration (I) or through the back conversion to trans-2-decanoyl-CoA, which is then converted to (III) and (IV) through Δ1-cis-Δ2-trans-enoyl-CoA isomerase. The equilibrium is in favor of the direction of forming 3-hydroxy metabolite (V), which can be metabolized further to lower chained fatty acids by the addition of NAD.

**Fig. 4.** A, the metabolism of cis-5-decenoic acid in the rat liver 10,000 x g pellet with the addition of ATP, CoA, and MgCl2 and incubated for 30 min at 37 °C. The metabolites were analyzed as trimethylsilyl derivatives on a capillary column gas chromatograph from 60 to 250 °C at 4 °C/min. The tracing shown is from the nonpolar SPB-1 column. The metabolites were identified by gas chromatography-mass spectrometry: 1, cis-5-decenoic; 2, cis-3-cis-5-decenoic; 3, trans-5-cis-5-decenoic; 4, trans-2-cis-5-decenoic; and 5, 3-hydroxy-cis-5-decenoic acids. B, the metabolism of cis-5-decenoic acid under the same conditions as A but with the additional NADPH. Under these conditions additional metabolites were detected. These metabolites are: 6, decanoic acid; 7, 3-hydroxydecenoic acid.

**Fig. 5.** Metabolism of cis-5-decenoic acid (I) in the rat liver 10,000 x g pellet with the addition of ATP, CoA, and MgCl2. The dehydrogenation to trans-2-cis-5-decenoyl-CoA (II) is significantly slower than the corresponding saturated decanoyl-CoA. Furthermore, the hydroxylated product, 3-hydroxy-cis-5-decenoyl-CoA (V), is not favorized in the equilibrium. Instead, the dienoyl-CoAs, which include cis-3-cis-5-decenoyl-CoA (III) and trans-3-cis-5-decenoyl-CoA as well as trans-2-cis-5-decenoyl-CoA, are predominant. Metabolites III and IV could be derived from II through the action of Δ1-cis-Δ2-trans-enoyl-CoA isomerase. The equilibrium concentration of V is too low to allow a detectable further metabolism through β-oxidation cycles even with the addition of exogenous NAD.

Similarly, the incubation of cis-5-dodecenoic acid for 30 min produced a metabolite profile consisting of cis-5-dodecenoic (62%), cis-3-cis-5-dodecadienoic (7%), trans-3-cis-5-dodecadienoic (7%), trans-2-cis-5-dodecadienoic (21%), and 3-hydroxy-cis-5-dodecenoic acids (1%). Likewise, the incubation
of cis-5-tetradecenoic acid produced a profile dominated by unreacted starting material (70%) and trans-2-cis-5-tetradecadienoic acid (23%), plus a minor amount of cis-3 and trans-3 di-unsaturated as well as 3-hydroxy metabolites. Unlike its saturated counterparts (Fig. 3) the equilibrium between 3-hydroxy fatty acids and the unsaturated metabolites favors the formation of unsaturated fatty acids (Fig. 5). The equilibrium ratio of 3-hydroxy metabolite to diunsaturated metabolites for cis-5-dodecanoic acid was 0.07 ± 0.05 after a 30-min incubation and decreased to 0.02 after a 120-min incubation. In contrast, the ratio for dodecanoic acid was 13 ± 2 in favor of the formation of 3-hydroxy metabolites. In addition, unlike the saturated counterparts the addition of NAD to the incubation mixture did not change the metabolite profile (data not shown). No cis-3-fatty acids, the postulated metabolic intermediate from the β-oxidation of cis-5 precursor, could be detected.

However, the addition of NADPH as shown in Fig. 4B changed the metabolite profile. cis-5-fatty acids were reduced to the corresponding saturated fatty acids, which then proceeded through the normal β-oxidation sequence to form 3-hydroxy fatty acids. The metabolite profile for cis-5-decanoic acid after a 30-min incubation in the presence of NADPH was starting material (75%), decanoic acid (15%), and 3-hydroxydecanoic acid (10%), plus some trace of trans-2-decanoic acid. For cis-5-tetradecenoic acid, the postulated metabolic intermediate in the β-oxidation of oleic acid, the profile changed similarly after the addition of NADPH (Fig. 6B): starting substrate (65%), tetradecanoic acid (23%), and 3-hydroxytetradecanoic acid (12%), plus a trace of trans-2-tetradecenoic acid. The addition of NAD in addition to NADPH (Fig. 6C) produced a metabolite profile of saturated fatty acids of several cycles of β-oxidation. These metabolites included tetradecanoic (8%), dodecanoic (8%), decanoic (4%), and octanoic (1%) acids. Again, no cis-3-fatty acids could be detected.

Cis-4-decenoic Acid—The CoA ester of this acid is a postulated intermediate in the metabolism of linoleic acid (11, 13). Because the NADPH-dependent 2,4-dienoyl-CoA reductase-mediated pathway of its metabolism is well documented this compound was studied under the same conditions for comparison. Without exogenous NAD and NADPH this substrate yielded a metabolite profile consisting of unreacted cis-4-decenoic (75%), trans-2-cis-4-decadienoic (13%), trans-2-trans-4-decadienoic acids (13%), and a trace amount of 3-hydroxy acid (<1%) after a 30-min incubation. The addition of NAD did not change this metabolite profile. However, the addition of NADPH produced a new profile. In addition to the unreacted substrate, trans-3-decenoic (10%), trans-2-decenoic (3%), and 3-hydroxydecanoic acids (23%) were identified. No decanoic acid could be detected.

Cis-3-decenoic Acid—The incubation (for 30 min) of this acid produced a metabolite profile consisting of unreacted cis-3-decenoic (19%), trans-2-decenoic (4%), and 3-hydroxydecanoic acids (74%). This profile is consistent with a Δ¹-cis-Δ²-trans-enoyl-CoA isomerase-mediated isomerization to trans-2-decenoic acid, then the normal β-oxidation sequence. However, the addition of NADPH facilitated the metabolic disposal of cis-3-decenoic acid to about the same rate as decanoic acid. In addition to the isomerase pathway, the cis-

![Fig. 6. A, the metabolism of cis-5-tetradecenoic acid in the rat liver 10,000 x g pellet with the addition of ATP, CoA, MgCl, and incubated at 37 °C for 30 min. The metabolites were analyzed as trimethylsilyl derivatives on a capillary column gas chromatograph from 60 to 250 °C at 4 °C/min. The metabolites are: 1, cis-5-tetradecenoic acid; 2, cis-3-cis-5-tetradecadienoic acid; 3, trans-3-cis-5-tetradecadienoic acid; 4, trans-2-cis-5-tetradecadienoic acid; 5, 3-hydroxy-cis-5-tetradecenoic acid. The major component of peak 5 was palmitoleic acid; however, in second intermediate polar SPB-35 capillary column (not shown), these two metabolites are separated to permit a quantitative determination. B, the same condition as A except that exogenous NADPH was added. New metabolites were formed: 6, tetradecanoic acid; and 7, 3-hydroxytetradecanoic acid. Peak 7 contains mostly palmitic acid, but separation can be achieved from the second intermediate polar SPB-35 column. C, the same condition as B except that NAD was also added. Additional metabolites from several cycles of β-oxidation from tetradecanoyl-CoA were produced. These metabolites are identified as: 8, octanoic acid; 9, decanoic acid; and 10, dodecanoic acid. No cis-3-docosenoic or any unsaturated docosenoic acids could be detected.
3 double bond was also reduced directly to form decanoic acid, which then underwent β-oxidation directly. With NADPH, the metabolite profile consisted of decanoic (24%), trans-2-decanoic (4%), and 3-hydroxydecanoic (68%) acids after a 30-min incubation.

Cis-7-dodecenoic Acid—To investigate whether the direct reduction of double bond in fatty acids also extends to the more remote cis-7 double bond and whether this more remote unsaturation affects the metabolic rate, the metabolism of cis-7-dodecenoic acid was studied. Without exogenous NAD and NADPH as co-factors the metabolic rate of cis-7-dodecenoic acid was not different from that of dodecanoic acid. After a 30-min incubation in a freshly prepared 10,000 x g pellet, the metabolite profile consisted of 3-hydroxy-cis-7-dodecenoic (62%), trans-2-cis-7-dodecenoic (5%), as well as cis-5-dodecenoic acids (10%) and its metabolites, which included trans-2-cis-5-dodecenoic (10%), cis-3-cis-5-dodecenoic, and trans-3-cis-5-dodecenoic acids. No 3-hydroxyoctanoic or unsaturated octenoic acids could be detected. The addition of NAD to the incubation decreased the amount of 3-hydroxy-cis-7-dodecenoic acid (25%) and increased the contribution of cis-5-dodecenoic acid (50%). Differing from the studies with dodecanoic acid, no lower chain fatty acids than cis-5-decenoic acid could be detected. The addition of NADPH yielded a profile that consisted of 3-hydroxy-cis-7-dodecenoic (47%) and cis-5-dodecenoic (21%) acids. In addition, 3-hydroxydecanoic acid (11%) was formed. Neither dodecanoic nor 3-hydroxydodecanoic acid was formed. These results show that with a double bond more remote than cis-5, the first cycle of β-oxidation is not affected in terms of the rate and the equilibrium ratio of 3-hydroxy to trans-2 metabolites. However, the metabolic conversion terminated at the second cycle and resulted in the accumulation of cis-5-dodecenoic acid and its unsaturated metabolites. The addition of NADPH did not reduce the cis-7 double bond directly; instead, the double bond was reduced at the stage of cis-5-dodecenoic acid to decanoic acid, which then was metabolized to 3-hydroxydecanoic acid. Therefore, the reduction pathway is active only on 3- and 5-enoyl-CoA but not the more remote double bonds.

Cis-9-dodecenoic Acid—The effect of an even more remote double bond at cis-9 in cis-9-dodecenoic acid was also studied. This substrate is also an n-3 fatty acid, which is an important class of fatty acids. As expected, the metabolic rate was not different from that of dodecanoic or cis-7-dodecenoic acid. In addition the metabolite profile was that which would be predicted from normal β-oxidation. The addition of NAD in the incubation extended the β-oxidation to cis-7-dodecenoic acid (18%), 3-hydroxy-cis-7-dodecenoic acid (21%), and a monounsaturated octenoic acid (4%) which is presumed to be cis-5-octenoic acid. Because octenoic acid is volatile the actual amount of octenoic acid produced could be much larger. When NADPH was added, octanoic acid was identified instead of unsaturated octenoic acid. No saturated dodecanoic or decanoic acid was produced when NADPH was included in the incubation. These results reaffirmed the notion that a remote double bond, such as cis-7 or cis-9, is not reduced by the reduction pathway; also, without NADPH, the β-oxidation terminated at a cis-5 intermediate.

Oleic Acid—From the above data we expected that the metabolism of oleic acid under the condition of limited NADPH should result in the accumulation of cis-5-tetradecenoic-CoA. Oleoyl-CoA was studied in the presence of NAD to sustain several cycles of β-oxidation. Cis-5-tetradecenoic acid was produced. Other metabolites detected were all intermediates prior to cis-5-tetradecenoic-CoA; these included 3-hydroxyoleate and an unsaturated hexadecenoic acid, presumed to be cis-7-hexadecenoic acid. No cis-3-dodecenoic acid or any intermediates with less than 14 carbons could be found. In contrast, when stearoyl-CoA was incubated under the same conditions dodecanoic acid was identified. These data together with those obtained in experiments with cis-7 and cis-9 dodecenoic acids indicate that a cis-5 double bond encountered during β-oxidation cycles has the same NADPH dependence as a terminal cis-5 double bond.

DISCUSSION

The metabolism of fatty acids in this 10,000 x g pellet preparation, which contains mitochondrial and peroxisomal enzymes, yielded 3-hydroxy fatty acids. Depending on whether the preparation is used fresh or is thawed from the frozen pellets, the β-oxidation can proceed through several cycles or stop in the first cycle at 3-hydroxy fatty acyl intermediates. The freezing and thawing process does not change the initial metabolic rate of fatty acids; it only limits the metabolism to the first cycle of β-oxidation through dehydrogenation and hydration. The possible reason could be that the freezing and thawing process disrupts the arrangement of fatty acid metabolic enzymes (19). Another possibility is that NAD, the limiting co-factor in the preparation, is diluted into a larger space after the disruption of organelles by freezing and thawing. In the frozen and thawed preparations, the sustained metabolism through more than one cycle of β-oxidation can be induced by the addition of exogenous NAD. The metabolite pattern obtained without exogenous NAD is similar to that found in intact mitochondria (20, 21) and in apparently intact peroxisomes (22) under conditions of limited NAD when these organelles are incubated with exogenous fatty acids as substrates. For experiments using saturated fatty acids as substrates 3-hydroxy fatty acids are the major metabolites together with some trans-2 precursor as well as cis-3 and trans-3 metabolites. 3-Unsaturated fatty acyl metabolites with unspecified double bond geometry together with trans-2- and 3-hydroxy fatty acids have been described by Stanley and Tubbs (20) in rotenone-inhibited mitochondriaoxidizing palmitoylcarnitine. The 3-ununsaturated fatty acyl metabolites are actually a mixture of cis-3 and trans-3 isomers.

For decades the metabolism of an even-numbered double bond in fatty acids was thought to be through the sequence of cis-4 → cis-2 → d-3-hydroxy- → L-3-hydroxy-fatty acyl-CoA (11). The last step of conversion is through the action of an epimerase. Recent data show that the major pathway is actually through an NADPH-dependent 2,4-dienoyl-CoA reductase from cis-4 → cis-4-trans-2 → trans-3 → trans-2 (11). The original 3-hydroxyacyl-CoA epimerase pathway is absent in mitochondria and plays only a minor role in peroxisomes of rat liver (23). In addition, the epimerization reaction is carried out by the concerted action of two enoyl-CoA hydratases instead of one single epimerase (16, 17). This NADPH-dependent 2,4-dienoyl-CoA reductase-mediated pathway of cis-4-decenoyl-CoA metabolism was observed in our study condition.

The metabolism of an odd-numbered double bond in fatty acids was postulated to go through cis-5- → cis-3- → trans-2- enoyl-CoAs (11). cis-3 fatty acid can be isomerized to trans-2-fatty acid as suggested by the conventional pathway (Fig. 7). However, our data show that cis-5-fatty acid cannot be β-oxidized to a cis-3-fatty acid. The block could be in the hydration step mediated by enoyl-CoA hydratase. Like the analogous cis-4-trans-2- enoyl-CoA, the intermediate cis-5-trans-2-enoyl-CoA also has an equilibrium favoring the formation of dienoyl-CoA (Fig. 5). At equilibrium the ratio of 3-hydroxy to trans-2 metabolites is 13 for the metabolism of
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Dodecenedioic and decenedioic acids show predominantly a possibly mediated via an 5-enoyl-CoA reductase, to tetrade-cis-5 instead of a cis-3 structure (5). Since cis-3-dicarboxylic coenzyme A pathway (pathway A) are then continued. At least the conventional cis-3-dodecenoyl-CoA, which is then reduced by a NADPH-dependent reaction, through a reductive metabolic reaction that requires NADPH.

Different from 2,4-dienoyl-CoA reductase, which requires a 2,4-dienoyl-CoA as substrate and reduces it to trans-3-enoyl-CoA, this metabolic conversion reduces the cis-5 double bond. Unlike cis-3 dicarboxylic acids, which is then reduced by a NADPH-dependent reaction, trans-2-fatty acids are oxidized by two cycles of β-oxidation to acetyl-CoA isomerase from here, six more cycles of β-oxidation metabolize the oleic acid completely to acetyl-CoA. Although cis-3-dodecenoyl-CoA (V) can be β-oxidized as postulated in this route, the β-oxidation of cis-5-tetradecenoyl-CoA (II) to V does not occur because of an unfavorable equilibrium. Therefore, in rat liver this route does not operate. In the revised pathway (route A), cis-5-tetradecenoyl-CoA (II) is reduced by a NADPH-dependent pathway to tetrade-cenoyl-CoA (III). Once the double bond is removed, the intermediate (III) proceeds to seven more cycles of β-oxidation to acetyl-CoA.

FIG. 7. Proposed metabolic scheme of oleic acid by β-oxidation. Oleoyl-CoA (I) is oxidized by two cycles of β-oxidation to cis-5-tetradecenoyl-CoA (II). From this intermediate two routes are diverted. In the conventional pathway (route B), II is converted to cis-3-dodecenoyl-CoA (V) by one more cycle of β-oxidation. In turn, V is isomerized to trans-2-dodecenoyl-CoA by 4-cis-Δ-2-trans-enoyl-CoA isomerase. From here, six more cycles of β-oxidation metabolize the oleic acid completely to acetyl-CoA. Although cis-3-dodecenoyl-CoA (V) can be β-oxidized as postulated in this route, the β-oxidation of cis-5-tetradecenoyl-CoA (II) to V does not occur because of an unfavorable equilibrium. Therefore, in rat liver this route does not operate. In the revised pathway (route A), cis-5-tetradecenoyl-CoA (II) is reduced by a NADPH-dependent pathway to tetradecenoyl-CoA (III). Once the double bond is removed, the intermediate (III) proceeds to seven more cycles of β-oxidation to acetyl-CoA.

Saturated fatty acids whereas it is 0.7 for cis-5-fatty acids and less than 0.7 for cis-4-fatty acids. The further β-oxidation of cis-5-enoyl-CoA to a cis-3-enoyl-CoA does not occur even with the addition of NAD. Similar to the analogous 3-hydroxy-cis-4-decenoyl-CoA (24), the equilibrium concentration of 3-hydroxy-cis-5-enoyl-CoA is apparently too low to support a detectable activity of dehydrogenation by 3-hydroxyacyl-CoA dehydrogenase to continue the oxidation cycle to yield cis-3-enoyl-CoA.

The metabolic block of a cis-5 double bond can be removed through a reductive metabolic reaction that requires NADPH. Different from 2,4-dienoyl-CoA reductase, which requires a 2,4-dienoyl-CoA as substrate and reduces it to trans-3-enoyl-CoA, this metabolic conversion reduces the cis-5 double bond directly. We propose a sequence for the metabolism of a fatty acid which contains an odd-numbered double bond, such as in oleic acid, as shown in Fig. 7. Oleoyl-CoA is metabolized via two cycles of β-oxidation to cis-5-tetradecenoyl-CoA, which is then reduced by a NADPH-dependent reaction, possibly mediated via an 5-enoyl-CoA reductase, to tetrade-cenoyl-CoA (pathway A). The normal cycles of β-oxidation are then continued. At least the conventional cis-3-dodecenoyl-CoA pathway (pathway B) does not operate in rat liver. It is likely that in humans the 5-enoyl-CoA reduction pathway is also predominant since urinary dicarboxylic acids such as dodecenedioic and decenedioic acids show predominantly a cis-5 instead of a cis-3 structure (5). Since cis-3-dicarboxylic acids are also identified in human urine (5, 18), it was origin-

This revised metabolic scheme explains why fatty acids with a cis-5 double bond have a significantly lower metabolic rate than either saturated or unsaturated fatty acids with double bonds further away from the carboxyl end (6–9, 25). It also explains the induction of an increased oxidation rate of arachidonic acid, which has an initial cis-5 double bond, under conditions favoring the formation of NADPH (8, 9). However, it is in apparent contradiction to the notion that an equal or faster metabolic rate of oleic acid occurs in rat liver mitochondria or peroxisomes when compared with other saturated fatty acids (7–9, 26) and that the oxidation rate of oleic acid is NADPH independent (8, 9). The explanation for this apparent discrepancy is that the conventional techniques used for the determination of oxidation rates depend only on the first one or two cycles of β-oxidation before the encounter of a cis-4 or cis-5 double bond; this is especially true when the oxidation is assessed with the measurement of radioactive acid-soluble product formation from the metabolism of 1-14C-labeled fatty acid substrates (7, 26). Significantly lower oxidation rates are obtained only on those fatty acids, such as γ-linolenic, arachidonic, eicosapentenoic, and docosahexaenoic acids (6–9), in which a cis-4 or cis-5 double bond is encountered in the first or after only one cycle of β-oxidation. Other supporting evidence is that the oxidation rates of linoleic and linolenic acids are also found to be independent of NADPH. Both linoleic and linolenic acids contain even-numbered double bonds, which require NADPH-dependent 2,4-dienoyl-CoA reductase for its metabolism. The “apparent” NADPH-independent metabolism observed in these unsaturated fatty acids as well as oleic acid must be an artifact resulting from measuring only the initial rates. In addition, the oxidation rate measured by oxygen consumption apparently falls to a slower rate with time (14) when the carnitine ester of unsaturated fatty acids is used as a substrate. The initial rate was used for comparison. Therefore, the metabolism of unsaturated fatty acids has to be reassessed with particular attention to the formation of products using chromatographic techniques instead of indirect experiments in which radioactive acid-soluble products are measured or in which NADH formation is measured.

The metabolism of a 3-enoyl-CoA can proceed through the isomerase pathway or the reduction pathway. Whether the reduction of the 3-enoyl-CoA is mediated by the same enzyme that reduces 5-enoyl-CoA or by another different enzyme is not known. In addition to 2,4-dienoyl-CoA reductase, apparently mitochondria and/or peroxisomes could contain other reductases that are specific for 3 and 5 double bonds without any activity on more remote double bonds. Since a 3-enoyl-CoA cannot be obtained from the β-oxidation of 5-enoyl-CoA, the biological function of Δ-3-trans-enoyl-CoA isomerase appears to be in the metabolism of even-numbered double bond instead of the odd-numbered double bond as suggested in the conventional pathway. This revised pathway for the
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Reductive metabolism of a cis-5 double bond in fatty acids may be also responsible for the β-oxidation of prostaglandins and related compounds since in some recent publications (27, 28) the metabolites identified are more consistent with a direct reduction of the cis-5 double bond.

REFERENCES