Hypertriglyceridemia and Fatty Liver of Fasting Rats after Administration of Emeriamine

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Summary The effect of emeriamine, a potent inhibitor of the entry of fatty acids into mitochondria on lipid metabolism, was examined. Emeriamine (10 mg/kg body weight) was orally administered to rats under the two different physiological conditions of a 2-day fast or refeeding with a high-carbohydrate diet after a 2-day fast. When rats were refed with a high-carbohydrate diet, serum and hepatic ketone bodies and the levels of free fatty acids decreased, and triglycerides significantly increased compared with fasting rats. However, no significant effect of emeriamine on serum and hepatic lipids was observed between two refeeding groups with or without emeriamine. Conversely, when emeriamine was administered to fasting rats, the levels of serum and hepatic triglycerides increased about 11- and 5-fold, respectively. However, the increased level of hepatic triglycerides was not accompanied by the activities of fatty acid synthetase and NADPH-generating enzymes. The analysis of serum lipoprotein revealed that very low-density lipoprotein consisted of triglyceride-rich particles and there were less apolipoproteins in the fasting rat given emeriamine. We also determined the 120-kDa protein content, which was probably dependent on lipogenesis. The level of 120-kDa protein was greatly increased with or without the administration of emeriamine after refeeding with a high-carbohydrate diet, but the concentration of 120-kDa protein was slight in the fasting rat with emeriamine. These results suggest that specific inhibition of fatty acid oxidation by emeriamine diverted the exogenous fatty acid to the esterification pathway, and induced fatty liver and hypertriglyceridemia under fasting conditions.

Key Words emeriamine, fatty liver, hypertriglyceridemia, lipoproteins, triglyceride

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Fatty acids used as alternative pathways of oxidation and esterification in the liver are mainly derived from plasma free fatty acids, from de novo synthesis of fatty acids, and from intrahepatic lipolysis (1, 2). The relative contributions from each source in the liver are variable and are under the influence of physiological conditions, such as hormones and nutrients (3). The concentrations of serum and hepatic lipids reflect the relative contribution of these pathways to some extent. However, it is not understood how to evaluate these contributions.

Inhibitors of fatty acid oxidation have been useful in the investigation of the relationship between fatty acid oxidation and lipid levels in the liver under various nutritional conditions. Kanamaru et al. (4, 5) discovered emeriamine, a derivative of a carnitine analogue from fungus (Emericella quadrilineata); this drug strongly inhibited the oxidation of fatty acids in homogenates of rat liver but was less effective in homogenates of heart and muscle.

We recently found increased amounts of the 120-kDa protein in the liver cytosol of genetically obese Zucker rats and also a transitory increase of the 120-kDa protein even in lean rats when refeeding with a high-carbohydrate diet after fasting. However, the physiological significance of the 120-kDa protein and how the levels of this protein are regulated remain uncertain (6, 7).

With the use of emeriamine, the present study investigated the integration of the metabolism of hepatic lipids that underlie the synthesis and secretion of triglycerides in fasting and fasted-then-fed rats, and the involvement of the 120-kDa protein under the condition of abnormal lipid metabolism.

MATERIALS AND METHODS

Animals. Male Wistar rats (5 or 6 weeks old) were fed standard laboratory chow ad libitum for 10 days to permit them to adjust to the new environment. Two experiments were conducted. In Experiment 1, rats weighing 175 to 195 g were divided into three groups. The first group was fasted for 48 h. The second and third groups were also fasted for 48 h and then were fed a high-carbohydrate diet (casein, 20%; sucrose, 10%; corn starch, 61%; soybean oil, 4%; mineral mixture, 4%; vitamin mixture, 1%) for 2 days. All three groups of rats were lightly anesthetized with ethyl ether, after which groups 1 and 2 received an oral administration of saline solution (2.5 ml/kg body weight), and group 3 received emeriamine (10 mg emeriamine/2.5 ml saline/kg body weight). After administration of saline or emeriamine, all three groups were fasted for 8 h before sacrifice. In Experiment 2, rats weighing 213 to 250 g were divided into two groups. After fasting for 48 h, they received an oral administration of saline solution or emeriamine in saline solution 8 h before sacrifice.

Enzyme assay. The livers were immediately removed and homogenized in a Teflon homogenizer with 10 volumes of 0.25 M sucrose solution containing 5 mM Tris-HCl buffer (pH 7.4) and 0.1 mM EDTA. The homogenate was centrifuged at 8,000 g for 20 min, and the supernatant was further centrifuged at 10,500 g for 60
min. The resultant supernatant was used as a source of enzymes. The activities of glucose-6-phosphate dehydrogenase [EC 1.1.1.49] (8), malic enzyme [EC 1.1.1.40] (9), and fatty acid synthetase [EC 2.3.1.85] (10) in hepatic cytosol fractions were determined photometrically. The enzyme activities were measured as mU/mg protein, where 1 mU is defined as the consumption of 1 μmol of NADPH per minute for fatty acid synthetase, or the formation of 1 μmol of NADPH for glucose-6-phosphate dehydrogenase and malic enzyme.

**Immunoblotting analysis of the 120-kDa protein.** SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of liver cytosol protein using 6% acrylamide (10 cm × 10 cm) was performed according to the method of Laemmli (11). Liver cytosol proteins corresponding to 35 μg protein were dissolved in a solution of 10% glycerol, 2.3% SDS, 62 mM Tris-HCl (pH 6.8), which contained 5% mercaptoethanol. Each gel electrophoresis was performed with three different amounts of the purified 120-kDa protein corresponding to 21.3 ng, 42.5 ng, and 85 ng as a standard calibration. After electrophoresis, the proteins on the gels were transferred to nitrocellulose paper, using a constant current of 120 mA for 1 h. The 120-kDa band was detected by using the rabbit 120-kDa antisera as the first antibody (7) and was visualized by an indirect immunoperoxidase assay. The scanning of the band by 4-chloro-l-naphthol was performed with a laser densitometer (Ultrascan 2202, LKB, Sweden) at 633 nm. The area of the 120-kDa protein peak was calculated by gravimetry.

**Lipid analysis.** Hepatic lipids were extracted and purified according to the method of Folch et al. (12). The extracts were then evaporated to dryness in a rotary evaporator, and the lipids were dissolved in petroleum ether. Hepatic triglyceride (13), phospholipid (14), and cholesterol (15) were determined colorimetrically. Serum triglyceride (16), phospholipid (17), cholesterol (18), and free fatty acids (19) were determined using an enzymatic kit (Wako Pure Chemicals Co., Osaka).

**Separation of lipoprotein.** Serum very low-density lipoprotein (VLDL, d < 1.006 g/ml) was isolated by ultracentrifugation after layering with aqueous NaCl (d = 1.006 g/ml) containing 0.05% EDTA at 15°C.

**Other measurements.** Serum ketone bodies were measured (20). The protein concentration was determined according to the method of Lowry et al. (21). The data were statistically evaluated by Student's t-test.

**RESULTS**

**Effect of emeriamine on serum and hepatic lipids and ketone body**

Table 1 shows the effects of emeriamine on serum and hepatic lipids after a 2-day fast followed by feeding for 2 days. After feeding with a high-carbohydrate diet, serum triglycerides, serum phospholipids, and hepatic triglycerides were increased approximately two- to three-fold compared with that of fasting rats. However, serum-free fatty acid, hepatic phospholipid, and cholesterol were signifi-
Table 1. Effects of emeriamine on the content of serum and hepatic lipids and ketone bodies after fasting followed by refeeding with a high-carbohydrate diet (Experiment 1).

|                      | Fasted saline | Fasted-refed saline | Fasted-refed emeriamine |
|----------------------|---------------|---------------------|------------------------|
| Serum                |               |                     |                        |
| Triglycerides (mg/dl)| 62.6±20.1     | 139±47*             | 136±34*                |
| Phospholipids (mg/dl)| 78.7±20.2     | 142±14**            | 146±17**               |
| Cholesterol (mg/dl)  | 72.2±14.5     | 78.4±1.2            | 72.0±9.7               |
| Free fatty acids (mEq/liter) | 1.18±0.19 | 0.57±0.15**         | 0.58±0.14**            |
| Ketone bodies (μmol/liter) | 63.4±23.4 | 1.30±0.96**         | 1.10±0.58**            |
| Liver                |               |                     |                        |
| Liver weight (g)     | 4.88±0.26     | 12.2±1.9**          | 12.2±0.9**             |
| Triglycerides (mg/g liver) | 10.9±2.7 | 37.5±11.0**         | 31.7±6.6**             |
| Phospholipids (mg/g liver) | 38.2±1.3 | 23.0±2.8**          | 21.3±1.6**             |
| Cholesterol (mg/g liver) | 3.71±0.41 | 2.35±0.20**         | 2.20±0.16**            |

Values are M±SD of five rats. The statistical significance of differences was compared with the fasted group and was calculated using Student’s t-test (*p<0.01, **p<0.001).

Effect of emeriamine following fasting and refeeding

Significantly decreased by refeeding. On the other hand, the increased serum ketone body resulting from fasting was greatly decreased by refeeding. However, no significant effect of emeriamine on serum, hepatic lipids, or ketone body was observed between the groups that were refed with or without emeriamine.

Table 2 shows the effects of emeriamine on serum and hepatic lipids after a 2-day fast. In the group in which emeriamine was administered 8h before sacrifice, the level of serum triglyceride increased approximately 11-fold and manifested milky hyperlipidemia. In addition, the level of hepatic triglycerides was also increased approximately five-fold, and a fatty liver was noted. Serum free fatty acids were further increased in the emeriamine-treated group than in the fasting group. The increase in serum ketone bodies caused by fasting was greatly attenuated in both fasted-refed groups with or without emeriamine (Table 1).

Effect of emeriamine on lipogenic enzymes

Table 3 shows the enzyme activity of malic enzyme, glucose-6-phosphate dehydrogenase, and fatty acid synthetase. Although each enzyme activity was greatly increased by feeding to the fasting rats, no significant difference in enzyme activity was observed between the two groups of refed rats, with or without the administration of emeriamine. In addition, the administration of emeriamine had no effect on enzyme activity in the fasting groups.

Effect of emeriamine on lipoprotein

The lipoprotein compositions of serum VLDL (d < 1.006 g/ml) isolated from
Table 2. Effects of emeriamine on the content of serum and hepatic lipids and ketone bodies of rats after a 2-day fast (Experiment 2).

|                      | Fasted saline       | Fasted emeriamine  |
|----------------------|---------------------|--------------------|
| Serum                |                     |                    |
| Triglycerides (mg/dl)| 44.3±7.4            | 500±167**          |
| Phospholipids (mg/dl)| 86.2±8.6            | 128±39             |
| Cholesterol (mg/dl)  | 70.7±7.4            | 68.0±20.5          |
| Free fatty acids (mEq/liter) | 0.97±0.13   | 3.57±0.37**        |
| Ketone bodies (μmol/liter) | 51.4±16.1        | 2.40±1.34**        |
| Liver                |                     |                    |
| Liver weight (g)     | 6.06±0.57           | 6.82±0.52          |
| Triglycerides (mg/g liver) | 14.2±3.4        | 81.1±1.6**         |
| Phospholipids (mg/g liver) | 37.9±1.5         | 36.4±0.9           |
| Cholesterol (mg/g liver) | 3.58±0.26        | 3.36±0.36          |

Values are M±SD of five rats. The statistical significance of differences was compared with fasted group and was calculated using Student’s t-test (**p<0.001).

Table 3. Effects of emeriamine on the activity of hepatic malic enzyme, glucose-6-phosphate dehydrogenase, and fatty acid synthetase.

|                      | Malic enzyme (mU/mg protein) | Glucose-6-phosphate dehydrogenase | Fatty acid synthetase |
|----------------------|-------------------------------|-----------------------------------|-----------------------|
| Experiment 1         |                               |                                   |                       |
| Fasted saline        | 13.9±4.2                      | 13.7±1.8                          | 0.21±0.12             |
| Fasted-refed saline  | 82.6±14.1**                   | 150±41**                          | 32.2±10.9**           |
| Fasted-refed emeriamine | 79.5±17.8**                  | 181±47**                          | 35.0±5.9**            |
| Experiment 2         |                               |                                   |                       |
| Fasted saline        | 7.72±2.68                     | 8.57±3.05                         | 0.36±0.29             |
| Fasted emeriamine    | 7.46±0.97                     | 8.36±3.30                         | 0.53±0.20             |

Values are M±SD of five rats. The statistical significance of differences was compared between the fasting groups of each experiment and was calculated using Student’s t-test (**p<0.001).

the fasted group, with or without emeriamine are shown in Table 4. The percentage of triglycerides was increased and that of protein was decreased in VLDL in the fasted group with emeriamine compared to the fasted group without emeriamine. However, no significant difference was seen in the percentages of phospholipid and cholesterol. These results indicate that VLDL was secreted from the liver into the bloodstream as triglyceride-rich particles.

**Effect of the concentration of 120-kDa protein**

Immunoblotting analysis with the 120-kDa protein antiserum was used to assess the content of 120-kDa protein in the hepatic cytosol protein (Fig. 1 and Table 5). When rats were starved for 2 days, the concentration of the 120-kDa
Table 4. The lipoprotein compositions of serum VLDL from fasted groups with or without emeriamine.

|                | Triglycerides | Phospholipids (%) | Cholesterol | Protein |
|----------------|---------------|-------------------|-------------|---------|
| Fasted saline  |               |                   |             |         |
| d < 1.006      | 44.9±4.1      | 25.4±1.6          | 12.0±4.7    | 17.7±1.6|
| Fasted emeriamine | 67.5±6.5**  | 17.4±5.7          | 7.0±1.7     | 8.1±1.1**|

Values are M±SD of five rats. The statistical significance of differences was calculated using Student's t-test between fasting groups with saline or emeriamine (**p<0.001).

Fig. 1. Immunoblotting analysis of the 120-kDa protein. Aliquots of hepatic cytosol proteins (35 μg of proteins) were analyzed by SDS-polyacrylamide gel electrophoresis (6% gel), and the bands were transferred to nitrocellulose paper and visualized by immunostaining. Each value represents one of five analyses. The arrow indicates the location of the 120-kDa protein. 1 and 4, Fasted; 2, Fasted-refed high-carbohydrate diet; 3, Fasted-refed high-carbohydrate diet with emeriamine; 5, Fasted with emeriamine (see Table 5).

The protein in the hepatic cytosol was low (less than 0.01 μg/35 μg of cytosol protein) and could not be detected by laser densitometry. After refeeding with a high-carbohydrate diet, the concentration of the 120-kDa protein was greatly increased, with or without the administration of emeriamine. On the other hand, when emeriamine was administered to fasting rats, the concentration of the 120-kDa protein was still low, although the levels of both serum and hepatic triglycerides were greatly increased (Table 2).
Table 5. The concentration of the 120-kDa protein in hepatic cytosol proteins.

| Experiment 1 | (μg/35 μg cytosol protein) | Experiment 2 | (μg/35 μg cytosol protein) |
|--------------|-----------------------------|--------------|-----------------------------|
| Fasted saline | undetectable                | Fasted saline | undetectable                |
| Fasted-refed saline | 0.143±0.046**             | Fasted-refed emeriamine | 0.147±0.026**             |
| Fasted emeriamine | undetectable              |              | undetectable              |

Values are M±SD of five rats. The statistical significance of differences was calculated using Student’s t-test between the two fasting groups (**p<0.001). The 120-kDa protein (less than 0.01 μg/35 μg cytosol protein) was observed by immuno-staining, but could not be detected using laser densitometry (see Fig. 1).

DISCUSSION

The administration of emeriamine to fasting rats caused almost complete cessation of ketone body production for up to 8 h. These results are in agreement with Kanamaru et al. (4, 5) and Harano et al. (22), who showed that the antiketogenic effects of emeriamine at a dose of 10 mg/kg body weight lasted for more than 6 h. This inhibition of ketogenesis is probably accompanied by a blockage of the entry of fatty acids into mitochondria through inhibition of carnitine palmitoyltransferase I activity (5).

Under these metabolic abnormalities after administration of emeriamine to fasting rats, the levels of serum and hepatic triglycerides were greatly increased. However, hypertriglyceridemia and fatty liver were not accompanied by increased de novo synthesis of fatty acids or increased NADPH-producing enzymes. The activities of enzymes such as fatty acid synthetase, glucose-6-phosphate dehydrogenase and malic enzyme were minimal (Table 3).

However, when resuming feeding of rats with a high-carbohydrate diet after a 2-day fast, de novo synthesis of fatty acid was increased for both groups with or without emeriamine. Consequently, the levels of serum and hepatic triglycerides without emeriamine were increased two- to three-fold by refeeding. However, the effects of emeriamine on the activities of lipogenic-related enzymes, lipids components, and ketone bodies were not significant. These results suggest that fatty acids mobilized from adipose tissue into hepatic cytosol under fasting conditions become trapped because of the completely inhibiting effect of emeriamine on the entry of fatty acids into mitochondria (4, 5, 23). Then, the nonutilized fatty acids seem to be esterified preferentially into triglycerides, deposited as hepatic triglycerides, and secreted into the bloodstream as VLDL that consist of triglyceride-rich particles with less apolipoproteins. In comparing the relative proportions of rat apolipoprotein C-II and C-III-3 by basic polyacrylamide gel electrophoresis containing 8 M urea, apoVLDL obtained by administration of emeriamine contained relatively less apolipoprotein C-II than that of fasting rats (unpublished observation, Maeda, H.).

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It has been reported that a reciprocal relationship exists between the rates of ketogenesis and secretion of triglycerides in perfused rat livers under various nutritional and physiological conditions. Ide and Ontko (23) also observed that the direct inhibition of the oxidation of hepatic fatty acids by the addition of 2-tetradecylglycidate increased the secretion of VLDL-triglyceride in the perfused livers of fasting rats. Jenkins and Griffith (24) also reported that mice given acetyl-aminocarnitine, a potent inhibitor of carnitine acyltransferase, followed by fasting are found to exhibit a reversible accumulation of triglycerides in the liver. However, mice given this inhibitor but not starved did not show this effect (25). These results were consistent with those obtained with emeriamine in the present study. Conversely, Fukuda and Ontko (26) reported that the inhibition of the synthesis of fatty acids in perfused livers by 5-tetradecyloxy-2-furoic acid simultaneously stimulated ketogenesis and reduced the secretion of VLDL-triglyceride followed by the inhibition of long chain fatty acid oxidation.

Additionally, we have previously reported on the isolation and purification of a 120-kDa protein in the liver cytosol of obese Zucker rats, and this protein may be responsible for the increased lipogenesis (6, 7). In this study, the increased levels of serum and hepatic triglycerides in fasting rats that were later given emeriamine were accompanied by a negligible amount of the 120-kDa protein and low fatty acid synthetase activity, indicating that the 120-kDa protein is correlated with de novo fatty acid synthesis, and not the esterification of fatty acids.

The data from the present study suggest that the pool size of fatty acids in cytosol or the enzyme activities involved in the entry of fatty acids into mitochondria is one of the causes of a type of hypertriglyceridemia and fatty liver.

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REFERENCES

1) McGarry, J. D., and Foster, D. W. (1980): Regulation of hepatic fatty acid oxidation and ketone body production. *Ann. Rev. Biochem.*, 49, 395-420.
2) Heimberg, M., Olubadewo, J. O., and Wilcox, H. G. (1985): Plasma lipoproteins and regulation of hepatic metabolism of fatty acids in altered thyroid states. *Endocrine Rev.*, 6, 590-607.
3) Katsurada, A., Iritani, N., Fukuda, H., Matsumura, Y., Nishimoto, N., Noguchi, T., and Tanaka, T. (1990): Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of acetyl-CoA carboxylase in rat liver. *Eur. J. Biochem.*, 190, 435-441.
4) Kanamaru, T., Shinagawa, S., Asai, M., Okazaki, H., Sugiyama, Y., Fujita, T., Iwatsuka, H., and Yoneda, M. (1985): Emeriamine, an antidiabetic β-aminobetaine
derived from a novel fungal metabolite. *Life Sci.*, **37**, 217–223.

5) Kanamaru, T., and Okazaki, H. (1989): Emeriamine: a new inhibitor of long chain fatty acid oxidation and its antidiabetic activity, in Novel Microbial Products for Medicine and Agriculture, ed. by Demain, A. L., Somkuti, G. A., Hunter-Cevera, J. C., and Rossmoor, H. W., Society for Industrial Microbiology, Elsevier, New York, pp. 135–144.

6) Maeda, H., and Kasahara, K. (1993): Increased 120kDa protein in liver cytosol of genetically obese Zucker rats. *J. Nutr. Sci. Vitaminol.*, **39**, 365–372.

7) Maeda, H., and Kasahara, K. (1994): Isolation and localization of the 120kDa protein in the liver of genetically obese Zucker rats. *J. Biochem.*, **115**, 37–40.

8) Kornberg, A., and Horecker, B. L. (1955): Glucose-6-phosphate dehydrogenase, in Methods in Enzymology, Vol. 1, ed. by Colowick, S. P., and Kaplan, N. O., Academic Press, New York, pp. 323–326.

9) Ochoa, S. (1955): Malic enzyme: malic enzymes from pigeon and wheat germ, in Methods in Enzymology, Vol. 1, ed. by Colowick, S. P., and Kaplan, N. O., Academic Press, New York, pp. 739–753.

10) Nepokroeff, C. M., Lakshmanan, M. R., and Porter, J. W. (1975): Fatty acid synthase from rat liver, in Methods in Enzymology, Vol. 35, ed. by Lowenstein, J. M., Academic Press, New York, pp. 37–44.

11) Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.

12) Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957): A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497–509.

13) Fletcher, M. J. (1968): A colorimetric method for estimating serum triglycerides. *Clin. Chim. Acta*, **22**, 393–397.

14) Gomori, G. (1942): A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter. *J. Lab. Clin. Med.*, **27**, 955–960.

15) Sperry, W. M., and Webb, M. (1950): A revision of the Shoenheimer-Sperry method for cholesterol determination. *J. Biol. Chem.*, **187**, 97–106.

16) Spayd, R. W., Bruschi, B., Burdick, B. A., Dappen, G. M., Eikenberry, J. N., Esders, T. W., Figueras, J., Goodhue, C. T., LaRossa, D. D., Nelson, R. W., Rand, R. N., and Wu, T.-W. (1978): Multilayer film elements for clinical analysis: applications to representative chemical determinations. *Clin. Chem.*, **24**, 1343–1350.

17) Takayama, M., Itoh, S., Nagasaki, T., and Tanimizu, I. (1977): A new enzymatic method for determination of serum choline-containing phospholipids. *Clin. Chim. Acta*, **79**, 93–98.

18) Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W., and Fu, P. C. (1974): Enzymatic determination of total serum cholesterol. *Clin. Chem.*, **20**, 470–475.

19) Shimizu, S., Yasui, K., Tani, Y., and Yamada, H. (1979): Acyl-CoA oxidase from *Candida tropicalis*. *Biochem. Biophys. Res. Commun.*, **91**, 108–113.

20) Harano, Y., Kosugi, K., Hysou, T., Uno, S., Ichikawa, Y., and Shigeta, Y. (1983): Sensitive and simplified method for the differential determination of serum levels of ketone bodies. *Clin. Chim. Acta*, **134**, 327–336.

21) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
22) Harano, Y., Kojima, H., Kashiwagi, A., Tanaka, Y., Nakamura, T., Fujita, T., and Shigeta, Y. (1987): A new carnitine analogue for the correction of metabolic derangements in diabetes, in Recent Trends in Management of Diabetes Mellitus, ed. by Sakamoto, N., Alberti, K. G. M. M., and Hotta, N., Elsevier Science Publishers, pp. 567–571.

23) Ide, T., and Ontko, J. A. (1981): Increased secretion of very low density lipoprotein triglyceride following inhibition of long chain fatty acid oxidation in isolated rat liver. *J. Biol. Chem.*, 256, 10247–10255.

24) Jenkins, D. L., and Griffith, O. W. (1985): DL-Aminocarnitine and acetyl-DL-aminocarnitine: potent inhibitors of carnitine acyltransferases and hepatic triglyceride catabolism. *J. Biol. Chem.*, 260, 14748–14755.

25) Jenkins, D. L., and Griffith, O. W. (1986): Antiketogenic and hypoglycemic effects of aminocarnitine and acylaminocarnitines. *Proc. Natl. Acad. Sci. U.S.A.*, 83, 290–294.

26) Fukuda, N., and Ontko, J. A. (1984): Interactions between fatty acid synthesis, oxidation, and esterification in the production of triglyceride-rich lipoproteins by the liver. *J. Lipid Res.*, 25, 831–842.