

# Methyl 2-Tetradecylglycidate, An Orally Effective Hypoglycemic Agent that Inhibits Long Chain Fatty Acid Oxidation Selectively

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## SUMMARY

The oral hypoglycemic agents methyl 2-tetradecylglycidate (McN-3716) and 2-tetradecylglycidic acid (McN-3802), synthesized in an effort to design a specific inhibitor of long-chain free fatty acid (FFA) oxidation, were tested for their effects on rat hemidiaphragm oxidation of various substrates to CO<sub>2</sub>. When added *in vitro*, both compounds were more potent (100–1000 times) in inhibiting long-chain FFA oxidation than either  $\alpha$ -bromopalmitic or 4-pentenoic acid. When fasting normal or diabetic rats were pretreated with McN-3716, the ability of the diaphragms to oxidize palmitate-1-<sup>14</sup>C to <sup>14</sup>CO<sub>2</sub> incubated *in vitro* was also diminished. Closely related nonhypoglycemic analogs of McN-3716 failed to inhibit oxidation. The failure of McN-3716 to inhibit the oxidation by diaphragm of short-chain fatty acids, palmitoyl carnitine, glucose, glycolytic intermediates,  $\beta$ -hydroxybutyrate, succinate, or citrate when added *in vitro* or after treatment of rats suggests that glycolysis, the tricarboxylic acid cycle, and the intramitochondrial  $\beta$ -oxidation of fatty acids were not inhibited. While depressing FFA oxidation, McN-3716 increased the ability of hemidiaphragms from normal and diabetic rats to oxidize glucose-<sup>14</sup>C to <sup>14</sup>CO<sub>2</sub>. This supports an intimate relationship between FFA oxidation and glucose utilization in hemidiaphragm. The locus of this stimulation appears to be the glycolytic pathway. The earliest onset for the impairment of diaphragm muscle FFA oxidation and the rise of plasma FFA after treatment of fasting rats with McN-3716 preceded the stimulation of hemidiaphragm glucose oxidation and the lowering of plasma glucose and tissue glycogen stores. These results suggest that the carbohydrate changes are secondary to the inhibition of FFA oxidation. **DIABETES** 28:242–248, March 1979.

For many years, it was generally believed that diabetes mellitus was first and foremost a disorder of carbohydrate metabolism and that any associated disturbances of free fatty acid (FFA) metabolism were only secondary effects, necessitated by the need for some tissue fuel other than carbohydrate. However,

studies by Randle and co-workers<sup>1–4</sup> have demonstrated that disturbances of carbohydrate metabolism that characterize "diabetic muscle" could be reproduced almost completely in normal muscle by an increased oxidation of FFA and ketones. From these observations emerged the hypothesis referred to by many as Randle's "glucose-fatty acid cycle." The suggestion was then made that excessive FFA metabolism might be a key factor underlying the decreased glucose tolerance in diabetes and other diseases.<sup>1,3</sup>

Therefore, if the above hypothesis is correct, then one possible approach to the treatment of diabetes mellitus would be to correct the abnormally high rate of FFA oxidation. That such an approach might be useful was supported by the finding that hypoglycin, 4-pentenoic acid, (+)-decanoylcarnitine, and  $\alpha$ -bromo fatty acids that inhibit FFA oxidation produced hypoglycemia.<sup>4,5,6,7,8</sup> However, since these inhibitors were toxic,<sup>4,5,9</sup> inhibited enzymes unrelated to hypoglycemia,<sup>4,5,10,11</sup> or were not orally effective ( $\alpha$ -bromo fatty acids and (+)-decanoylcarnitine), this pharmacologic approach to diabetic treatment has not been fully tested.

Therefore, the fatty acid analog McN-3716, methyl 2-tetradecylglycidate, shown below was synthesized as part of a synthetic program directed at generating analogs of FFA that would be orally effective inhibitors of long-chain FFA oxidation. In this report, the potency and specificity of McN-3716 in inhibiting FFA oxidation will be described and evidence presented that this action of the drug results in enhanced glucose utilization. Elsewhere,<sup>12</sup> it has been reported that McN-3716 produces a dose-dependent oral hypoglycemic effect in normal and diabetic rats, mice, and dogs and to reverse the ketoacidosis of alloxan diabetic rats and depancreatized dogs.

Part of this data was presented at the 38th Annual Meeting of the American Diabetes Association, Boston, 1978, and at the Annual Meeting of the American Society of Biological Chemists, Atlanta, 1978.

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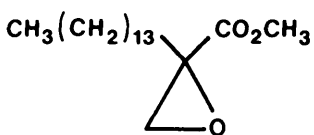
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**EXPERIMENTAL PROCEDURE**

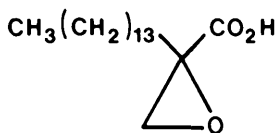
**Materials.** All radioactive substrates were purchased from New England Nuclear except for DL-hydroxy-3-<sup>14</sup>C butyric acid, which was purchased from Amersham Searle. Non-labeled substrates were purchased from either Sigma or Boehringer-Mannheim and were of the highest purity available. Hyamine was purchased from Packard Instrument, recrystallized bovine serum albumin from Sigma, fat-free albumin from Schwarz-Mann, and L-carnitine from General Biochemicals. 4-Pentenoic acid was obtained from K & K Laboratories and gave a single spot on TLC.  $\alpha$ -Bromopalmitic acid was purchased from Chemical Procurement Lab and was determined to be 95% pure by gas liquid chromatography (3% SE30 on Chrom Q; derived as methyl ester, using diazomethane).

Shown below are the structures of five fatty acid analogs that were used in these studies. The complete synthesis of these compounds will be reported in detail later. All were pure samples and were characterized satisfactorily by melting point, NMR, MS, IR, and elemental analysis.

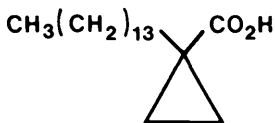
McN-3716 methyl 2-tetradecylglycidate



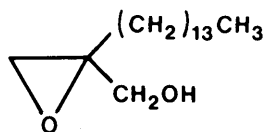
McN-3802 2-tetradecylglycidic acid



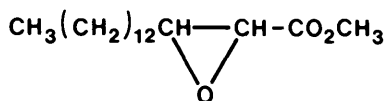
McN-3845 1-tetradecylcyclopropane carboxylic acid



McN-3841 2-tetradecyloxiranemethanol



McN-X-847 methyl trans-3-tridecylglycidate



**Methods.** Male albino Sprague-Dawley rats (150–280 g), purchased from Charles River and maintained on Wayne rat chow, were fasted for 24 h and given water ad libitum.

Methods for preparing hemidiaphragms, buffers, drug solutions, streptozotocin diabetic rats, and for analyzing <sup>14</sup>CO<sub>2</sub> formation, blood glucose, blood urea nitrogen (BUN), and FFA have been described elsewhere.<sup>13</sup>

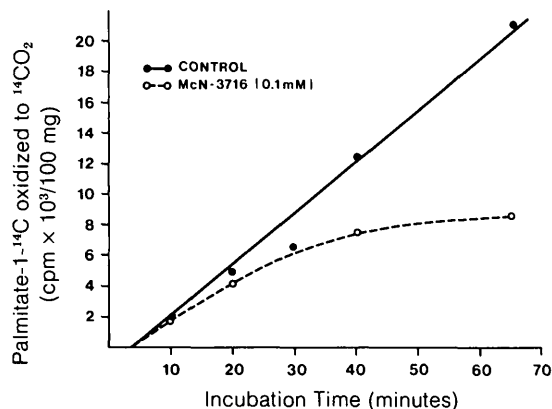
After preincubation for 20–30 min in ice-cold Krebs-Ringer phosphate buffer (KRP), the hemidiaphragms were blotted, weighed, and added to reaction flasks. The reaction flasks contained 2 ml of KRP with glucose-<sup>14</sup>C and fructose-U-<sup>14</sup>C (5 mM, 1  $\mu$ Ci),  $\beta$ -hydroxy-butyrate-3-<sup>14</sup>C (0.1 mM, 0.5  $\mu$ Ci), or other nonfatty acid substrates (2 mM, 0.5  $\mu$ Ci). For studies with fatty acids and palmitoylcarnitine, the tissues were added to flasks containing 3 ml of FFA-albumin solutions (0.3  $\mu$ mol FFA, 0.5  $\mu$ Ci). The FFA-albumin solutions were prepared by the method of Spector et al.<sup>14</sup> The resulting solutions contained approximately 1  $\mu$ mol bovine serum albumin per 5–7  $\mu$ mol FFA. The test compounds were added (100  $\mu$ l) to the final reaction mixture.

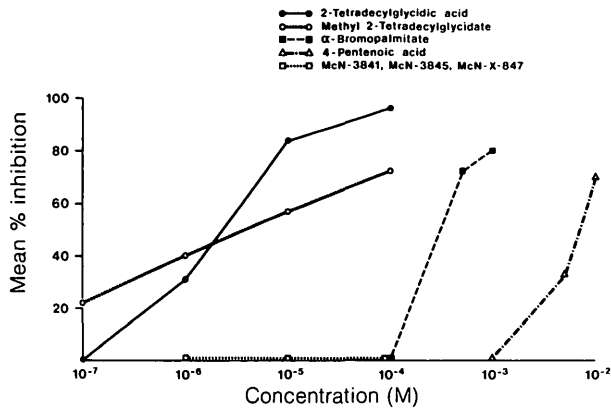
The flasks were equipped with two plastic wells, one containing a filter paper saturated with hyamine and the other containing 0.5 ml of 6N perchloric acid. Unless otherwise noted, all reaction vials were sealed after hemidiaphragm addition and incubated at 37°C for 60–120 min in a Dubnoff shaker (60 cycles/min). At the end of incubation, the acid was tipped from the sidearm, and shaking was continued for at least 1 h during which <sup>14</sup>CO<sub>2</sub> was quantitatively trapped in hyamine.

Kidney cortex slices (approximately 40 mg wet wt) from 48-h fasted rats and epididymal fat pads (approximately 100 mg) from 18–24 h fasted rats were studied under the same incubation conditions described for hemidiaphragms.

For experiments in which the animals were pretreated with the drugs, the diaphragms were incubated in the absence of drugs immediately without preincubation. The drugs were administered orally, suspended in 0.5% methyl cellulose (Fisher Scientific). It was found that the suspending agent was without effect on any parameter measured. Whole blood was collected in heparinized tubes, placed on ice, and plasma was obtained in a refrigerated centrifuge for immediate analysis of glucose, BUN, and FFA. One-gram samples of liver, whole gastrocnemii, and heart muscles were weighed, frozen, and stored at –4°C until analyzed for glycogen. The methods for isolation and hydrolysis of tissue glycogen have been published previously.<sup>15</sup> The glycogen hydrolysates were diluted to 10–25 ml and

**FIGURE 1.** Effect of McN-3716 on palmitate-1-<sup>14</sup>C oxidation to <sup>14</sup>CO<sub>2</sub> by isolated hemidiaphragms from fasted rats. All points were the mean of quadruplicate experiments.





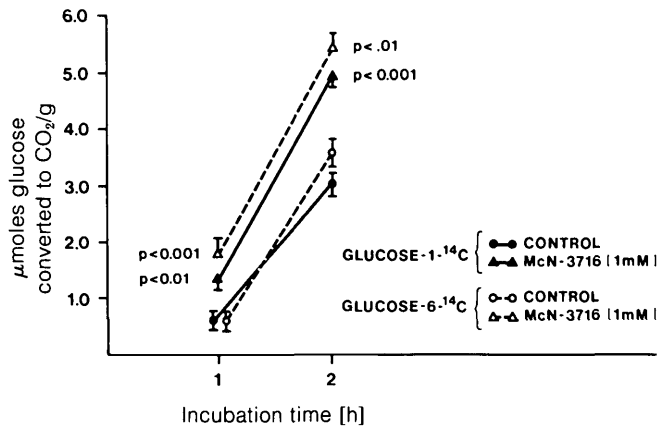
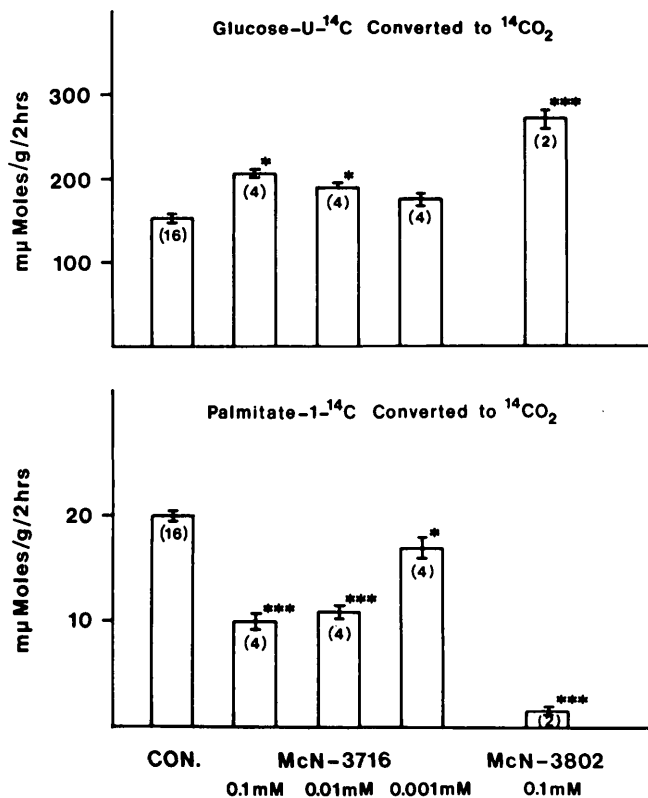
**FIGURE 2.** Effect of various fatty acid analogs on rat hemidiaphragm oxidation of palmitate-1-<sup>14</sup>C in vitro. The fatty acid analogs studied were McN-3802, McN-3716,  $\alpha$ -bromopalmitate, 4-pentenoic acid, and nonhypoglycemic analogs—McN-3841, McN-X-847, and McN-3845. All values were calculated compared with their own control contralateral hemidiaphragms. Drugs were added immediately before addition of tissues and incubation for 1 h.

duplicate 0.5–2.0 ml samples were taken for analysis of glucose using the Glucostat reagent (Worthington) prepared in 0.1M phosphate buffer, pH 7.0.

**RESULTS**

**Effects in vitro.** As shown in Figure 1, the addition of methyl-2-tetradecylglycidate (McN-3716) to isolated rat hemidiaphragms in vitro inhibited the rate of oxidation of palmitate-1-<sup>14</sup>C to <sup>14</sup>CO<sub>2</sub>. It should be noted that there was a lag before the onset of inhibition. If the tissues were first preincubated

**FIGURE 3.** Effect of McN-3716 and McN-3802 on rat hemidiaphragm oxidation of palmitate-1-<sup>14</sup>C and glucose-<sup>14</sup>C in vitro. Numbers in parenthesis are the numbers of tissues studied. Significance compared with controls determined by Student's *t* test. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001. Con, control tissues.



**FIGURE 4.** Effect of McN-3716 on the oxidation of glucose-1-<sup>14</sup>C and glucose-6-<sup>14</sup>C by isolated rat quarter-diaphragms. Each value is the mean of quadruplicate determinations. Significance compared with appropriate controls is shown beside the mean of McN-3716 response.

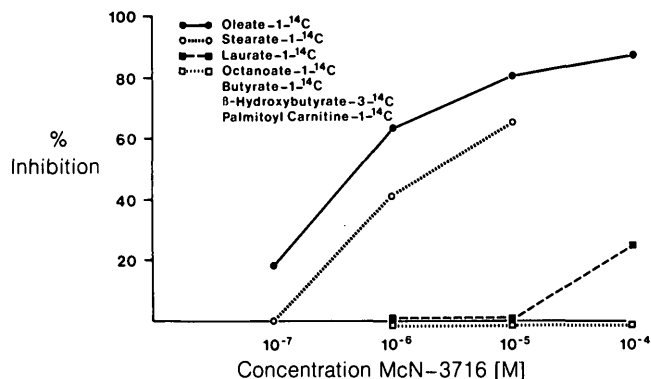
with McN-3716 for 20 min, then no lag phase was found. The inhibition of palmitate oxidation produced by both McN-3716 and its free acid, McN-3802, was concentration-dependent down to 10<sup>-6</sup>–10<sup>-7</sup>M. Examples of these testing results are shown in Figures 2 and 3. Also shown in Figure 2, for comparison, are results with the reported inhibitors of fatty acid oxidation,  $\alpha$ -bromopalmitic and 4-pentenoic acid, and several close structural analogs of McN-3716 (McN-3841, McN-X-847, and McN-3845) that do not produce hypoglycemia (manuscript in preparation).

At higher concentrations (10<sup>-5</sup>–10<sup>-3</sup>M), the direct addition of McN-3716 and McN-3802 to hemidiaphragms from fasted nondiabetic rats (Figure 3) accelerated the rate of glucose-U-<sup>14</sup>C oxidation. Furthermore, as shown in Figure 4, McN-3716 significantly increased the oxidation of glucose labeled in either the C-6 or C-1 positions by quarterdiaphragms from nondiabetic rats.

The effect of McN-3716 on the oxidation of other fatty acids is shown in Figure 5. McN-3716 exhibited greater potency in inhibiting the oxidation of oleate, stearate, and palmitate (see Figures 2 and 3) than of laurate and failed to inhibit the oxidation of palmitoylcarnitine or short-chain fatty acids. In other studies, it was found that McN-3716 (0.0001–0.1 mM) does not significantly (*P* < 0.05) inhibit or stimulate the oxidation by rat hemidiaphragm of 1,5-<sup>14</sup>C-citrate, 1,4-<sup>14</sup>C-succinate, 1-<sup>14</sup>C-lactate, or 1-<sup>14</sup>C-pyruvate.

Whether the effects of McN-3716 are reversible is still not completely clear. As shown in Figure 6, large amounts of unlabeled palmitic acid added simultaneously with McN-3716 protected against the approximately 50% inhibition of palmitate-1-<sup>14</sup>C oxidation produced by 10<sup>-5</sup>M McN-3716. However, when this experiment was repeated by first preincubating the tissues for 20 min at 37°C with 10<sup>-5</sup>M McN-3716, the inhibition was not completely reversible.

It was found that the ability of McN-3716 and McN-3802 to inhibit palmitate-1-<sup>14</sup>C oxidation was not specific for muscle. McN-3716 and McN-3802 also inhibited in vitro the oxidation of palmitate-1-<sup>14</sup>C to <sup>14</sup>CO<sub>2</sub> when isolated kidney cortex slices (Figure 7), epididymal fat pads (68–72% at 0.01–1 mM), and hepatocytes from rats fasted 24–48 h (26–80% at 0.0005–0.01 mM) were used. The inhibitory effect of McN-3716 and McN-3802 on fatty acid oxidation,



**FIGURE 5.** Effect of McN-3716 on the oxidation of various fatty acids by rat hemidiaphragm *in vitro*. All values were the mean of at least triplicate experiments, and percent inhibitions were calculated in comparisons to mean of all the control contralateral hemidiaphragms that were used in any one experiment (N = 4–10). All tissues were incubated for 60 min. The absolute values for the oxidation were used for determination of statistical significance. Results at all concentrations that are shown to inhibit the fatty acid oxidation were significantly lowered ( $P < 0.05$ ) compared with the controls as determined by the Student's *t* test.

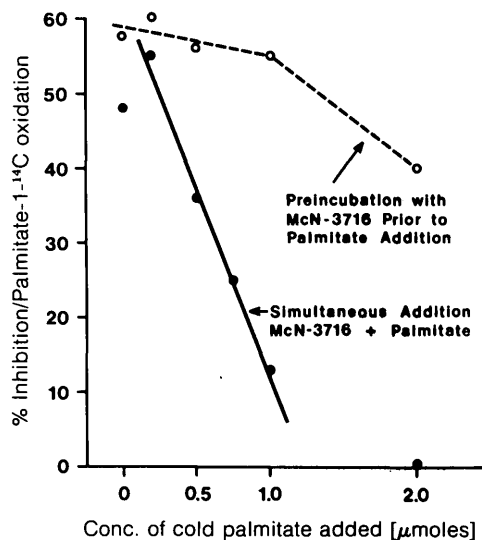
ketogenesis, and gluconeogenesis, tested by using hepatocytes will be reported elsewhere.<sup>16</sup>

**Effect of pretreatment of rats with McN-3716 and analogs on ability of hemidiaphragms to oxidize substrates *in vitro*.**

Hemidiaphragms removed from animals pretreated with McN-3716 were evaluated to determine if they have a depressed ability to oxidize palmitate-1-<sup>14</sup>C when incubated *in vitro*. The results of a typical experiment using a dose of 25 mg/kg, p.o. of McN-3716 are shown in Table 1. McN-3716 markedly inhibited FFA oxidation, stimulated glucose oxidation, elevated FFA, lowered plasma glucose, and depleted the liver and muscle glycogen stores of fasted rats. None of these metabolic perturbations could be reproduced at 25 mg/kg, p.o. by the nonhypoglycemic analogs of McN-3716, McN-X-847, or McN-3845. In other experiments using lower doses of McN-3716 (10–15 mg/kg, p.o.), it was found that the earliest changes after dosing (60 min) that were significantly different from controls were the elevation of plasma FFA and inhibition of oxidation of palmitate-1-<sup>14</sup>C by rat hemidiaphragm. At 120–180 min after dosing, hypoglycemia was apparent, BUN levels were elevated, and the capacity of the hemidiaphragm to oxidize glucose was enhanced. Furthermore, in contrast to results reported for hypoglycin and 4-pentenoic acid,<sup>17</sup> none of the above effects were reversed when fasting rats were given doses of L-carnitine (100 mg/kg, s.c.) immediately after and at 30 and 90 min after McN-3716 (20 mg/kg, p.o.) administration.

The effects of pretreatment of fasted nondiabetic rats with McN-3716 (25 mg/kg, p.o.) and vehicle on the ability of diaphragms to oxidize various other substrates was also studied. As shown in Table 2, McN-3716 accelerated the oxidation of not only glucose but also of fructose, fructose-6-PO<sub>4</sub>, fructose-1,6-diphosphate, β-glycerophosphate, and pyruvate. It did not significantly stimulate the ability of diaphragms to oxidize citrate-1,5-<sup>14</sup>C.

Consistent with the Randle theory, it has been reported<sup>12</sup> that McN-3716 lowers blood glucose under conditions where fatty acids were the major energy substrates (fasting and diabetes), but not under conditions where carbohydrate



**FIGURE 6.** Reversibility of McN-3716 inhibition of palmitate-1-<sup>14</sup>C oxidation *in vitro* by addition of nonradioactive palmitic acid. In one case (●—●), rat hemidiaphragms were preincubated for 20 min in the absence of McN-3716, as described in Methods. The hemidiaphragms from each rat were then blotted and incubated in buffer containing palmitate-1-<sup>14</sup>C (0.04 μmol–0.5 μCi), and various amounts of cold palmitic acid bound to bovine serum albumin (5–7 μmol/1 μmol of fat-free albumin) in the presence or absence of 10<sup>-5</sup>M McN-3716. Each point represents mean percent inhibition produced by McN-3716, using three pairs of hemidiaphragms. In the other experiment (○—○), the protocol was the same as above except hemidiaphragms were preincubated at 37°C in presence or absence of McN-3716. Drug was not added during the period when hemidiaphragms were incubated with the palmitic acid.

was the main energy source (fed state or after hypophysectomy). Furthermore, McN-3716 reverses the ketoacidosis of diabetic rats and depancreatized dogs.<sup>12</sup> Therefore, it was of interest to obtain more direct evidence in an experimental protocol similar to that described above on whether

**FIGURE 7.** Effect of McN-3716 on rat kidney cortex slice palmitate-1-<sup>14</sup>C oxidation *in vitro*. The numbers in parentheses are the number of tissues studied. Significance from control tissues determined by Student's *t* test; \*\*\* $P < 0.001$ . I, percent inhibition.

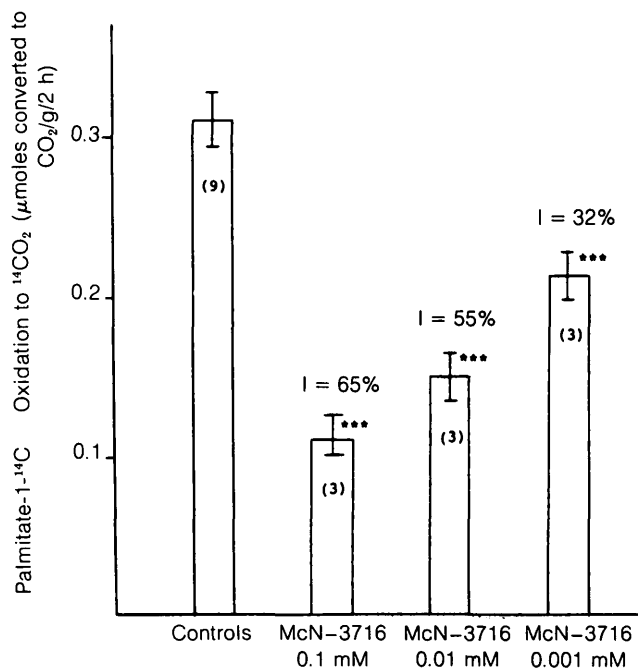


TABLE 1

Effect of pretreatment of fasted rats with McN-3716 at 25 mg/kg, p.o. and vehicle on plasma FFA, plasma glucose levels and on the ability of hemidiaphragm to oxidize FFA and glucose when incubated in vitro

Parameter measured	Treatment	Hours after treatment		
		0.5	1.0	2.0
Palmitate-1- <sup>14</sup> C oxidation $\mu$ mol converted g-h	Vehicle	0.042 $\pm$ 0.002*	0.054 $\pm$ 0.003	0.047 $\pm$ 0.03
	McN-3716	0.045 $\pm$ 0.003	0.044 $\pm$ 0.002†	0.024 $\pm$ 0.004‡
Glucose- <sup>14</sup> C (U) oxidation $\mu$ mol converted g-h	Vehicle	0.79 $\pm$ 0.03	0.85 $\pm$ 0.06	0.72 $\pm$ 0.03
	McN-3716	0.90 $\pm$ 0.08	1.15 $\pm$ 0.06†	1.49 $\pm$ 0.08§
Plasma FFA	Vehicle	869 $\pm$ 67	903 $\pm$ 18	997 $\pm$ 52
	McN-3716	1815 $\pm$ 134§	1987 $\pm$ 62§	2458 $\pm$ 28§
Plasma glucose (mg/dl)	Vehicle	98 $\pm$ 5	105 $\pm$ 6	104 $\pm$ 4
	McN-3716	87 $\pm$ 5	93 $\pm$ 4	86 $\pm$ 4†
Liver glycogen mg/g tissue	Vehicle	0.30 $\pm$ 0.03	0.41 $\pm$ 0.03	0.34 $\pm$ 0.03
	McN-3716	0.35 $\pm$ 0.03	0.34 $\pm$ 0.01	0.12 $\pm$ 0.02‡
Heart glycogen mg/g tissue	Vehicle	2.6 $\pm$ 0.2	2.4 $\pm$ 0.07	2.7 $\pm$ 1
	McN-3716	2.9 $\pm$ 0.2	2.5 $\pm$ 0.03	0.2 $\pm$ 0.04§
Gastrocnemius glycogen mg/g tissue	Vehicle	0.27 $\pm$ 0.01	0.23 $\pm$ 0.01	0.25 $\pm$ 0.01
	McN-3716	0.24 $\pm$ 0.02	0.24 $\pm$ 0.01	0.07 $\pm$ 0.01§

\* Mean  $\pm$  SEM, four rats per group. † P < 0.05, ‡ P < 0.01, § P < 0.001.

McN-3716 also inhibits the accelerated fatty acid oxidation of diabetes in animals. As shown in Figure 8, pretreatment of fasted streptozotocin diabetic rats with McN-3716 at 18 and 2 h before sacrifice significantly inhibited the ability of the hemidiaphragms to oxidize palmitate to CO<sub>2</sub>. The rates were lowered to the levels seen for diabetic rats fed ad libitum (see Figure 8). These rates were similar to those observed in other experiments that used nondiabetic rats (blood glucose 98–121 mg/dl). Also shown in Figure 8,

treatment of diabetic rats with McN-3716 doubled the ability of the hemidiaphragms to oxidize glucose and lowered the blood glucose into the normal range. These effects occurred without a significant elevation of FFA.

#### DISCUSSION

The evidence for and against the glucose-fatty acid cycle proposed by Randle and co-workers has been summarized in detail elsewhere.<sup>1,3,18,19</sup> Randle also proposed that, if

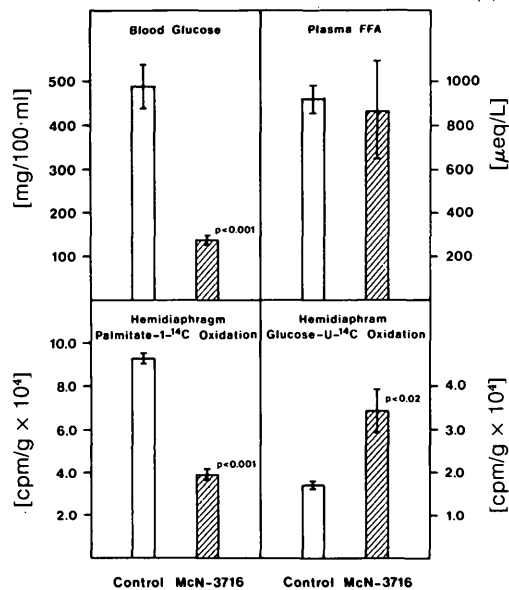
TABLE 2

Effect of pretreatment\* of fasted rats with McN-3716 (25 mg/kg, p.o.) and vehicle on the ability of the quarterdiaphragms to oxidize various substrates when incubated in vitro

Substrate	Treatment	$\mu$ moles converted to CO <sub>2</sub> /g/h	Percent stimulation
U- <sup>14</sup> C-Glucose	Vehicle	0.54 $\pm$ 0.04	78
	McN-3716	0.96 $\pm$ 0.07‡	
U- <sup>14</sup> C-Fructose	Vehicle	0.27 $\pm$ 0.02	111
	McN-3716	0.57 $\pm$ 0.02‡	
1- <sup>14</sup> C-Pyruvate	Vehicle	6.90 $\pm$ 0.32	26
	McN-3716	8.72 $\pm$ 0.62†	
1,5- <sup>14</sup> C-Citrate	Vehicle	0.33 $\pm$ 0.03	0
	McN-3716	0.34 $\pm$ 0.03	
U- <sup>14</sup> C-Fructose-6-PO <sub>4</sub>	Vehicle	0.30 $\pm$ 0.02	110
	McN-3716	0.63 $\pm$ 0.04‡	
U- <sup>14</sup> C-Fructose-1,6-diphosphate	Vehicle	0.59 $\pm$ 0.02	86
	McN-3716	1.10 $\pm$ 0.09‡	
U- <sup>14</sup> C- $\beta$ -Glycerophosphate	Vehicle	0.58 $\pm$ 0.03	116
	McN-3716	1.25 $\pm$ 0.08‡	
1- <sup>14</sup> C-Pyruvate	Vehicle	9.31 $\pm$ 0.63	54
	McN-3716	14.33 $\pm$ 0.46‡	

\* Two groups of six rats were pretreated with either drug or vehicle at 2 h before sacrifice. The diaphragms from each animal were divided into four equal parts that were each incubated in the presence of a different substrate.

Significance compared with controls determined by Student's *t* test. † P < 0.05; ‡ P < 0.01.



**FIGURE 8.** Effect of pretreatment of fasted streptozotocin diabetic rats with McN-3716 ( $2 \times 20$  mg/kg, p.o.) on glucose and FFA levels and ability of hemidiaphragms incubated *in vitro* to oxidize palmitate- $1\text{-}^{14}\text{C}$  and glucose- $^{14}\text{C}$ (U). Rats were dosed with McN-3716 at 18 and 2 h and fasted 18 h before sacrifice. Significance compared with vehicle-treated rats determined by Student's *t* test. Palmitate- $1\text{-}^{14}\text{C}$  oxidation by hemidiaphragms from fed rats (blood glucose  $859 \pm 98$  mg/dl) was  $38,638 \pm 3843$  cpm/100 mg tissue weight.

the release and oxidation of fatty acids make a significant contribution to the pathogenesis of clinical or experimental diabetes, then inhibitors of fatty acid oxidation should improve blood glucose utilization.<sup>4</sup> However, such inhibitors will have therapeutic potential only if they suppress fatty acid oxidation, but not if they have more general inhibitory effects on intermediary metabolism. As noted in the introduction, hypoglycin and 4-pentenoic acid produce hypoglycemia at high doses and inhibit palmitate oxidation; however, their effects on metabolism were not confined to fatty acid oxidation. Acyl-D-carnitines and  $\alpha$ -bromo fatty acids also inhibited fatty acid oxidation; however, they were toxic<sup>4,5,7,9,11</sup> and were not orally effective. Therefore, the compounds synthesized in our laboratories, McN-3716 and McN-3802, may be the first orally effective compounds with sufficient specificity to test the therapeutic possibilities proposed by Randle.

It was found that McN-3716 and McN-3802 were anywhere from 100–1000 times more potent in inhibiting fatty acid oxidation *in vitro* (Figure 2) and producing hypoglycemia<sup>12</sup> than either  $\alpha$ -bromopalmitic or 4-pentenoic acid. They did not, however, inhibit the oxidation by diaphragm of short-chain fatty acids, lactate, pyruvate, glucose,  $\beta$ -hydroxybutyrate, citrate, succinate, and glycolytic intermediates after their addition *in vitro* or after pretreatment of rats *in vivo*. Thus, the decreased  $\text{CO}_2$  formation from fatty acids cannot be explained as resulting from inhibition of glycolysis or the tricarboxylic acid cycle. Certainly, the actions of McN-3716 and McN-3802 are not the result of nonspecific interaction of fatty acids or epoxides with fatty acid oxidation, since over a hundred related fatty acid analogs,  $\alpha$ -substituted fatty acids, glycidates, and epoxides tested in our laboratory failed to inhibit at concentrations below 0.1 mM. While effects of other fatty acids will be reported later, it is reported here that even close structural analogs

(McN-3845, McN-3841, and McN-X-847) fail to inhibit when added *in vitro* (Figure 2) or to produce comparable metabolic perturbations when given *in vivo*. Furthermore, the observation that McN-3716 inhibited long-chain but not short-chain fatty acid- $^{14}\text{C}$  or  $1\text{-}^{14}\text{C}$ -palmitoylcarnitine oxidation (Figure 5) suggests that the site of inhibition is at a metabolic step before the  $\beta$ -oxidation of FFA since the pathways for  $\beta$ -oxidation of long- and short-chain FFA in the mitochondria are identical. While further experiments are in progress, studies<sup>16</sup> using isolated rat hepatocytes have suggested that the carnitine acyl transferase I is the site of inhibition.

Rudermen and co-workers<sup>18</sup> have pointed out that the inhibition by fatty acids of glucose metabolism in muscle other than heart is questionable. Randle et al.<sup>3</sup> obtained slight effects on glucose metabolism when FFA were added to diaphragms. Böhning<sup>19</sup> substantiated these findings, using supraphysiologic concentrations of FFA, while Schonfield and Kipnis<sup>20</sup> could detect no effect. However, all of these studies suffer from lack of evidence of whether the added fatty acids produced an increase in flux through FFA oxidation. However, when FFA oxidation of hemidiaphragms from normal or diabetic rats was inhibited with McN-3716 (Figures 3 and 8, Table 1), the ability of hemidiaphragms to oxidize glucose was enhanced. This finding thus supports an intimate relationship between FFA oxidation and glucose utilization in hemidiaphragm as well as in heart muscle. The earliest onset for the impairment of FFA oxidation and the rise of plasma FFA preceded the stimulation of glucose oxidation and the lowering of plasma glucose, thus suggesting that the changes in carbohydrate metabolism, including the depletion of glycogen, are secondary to the inhibition of fatty acid oxidation.

It was found that McN-3716 accelerates only the oxidation of substrates that enter the scheme of increased glucose metabolism at steps before the Krebs' cycle (did not inhibit citrate or succinate oxidation). This suggests that McN-3716 may be reversing the well documented<sup>1,3</sup> feedback inhibition of the glycolytic pathway and pyruvate dehydrogenase that occurs under conditions of high FFA oxidation. Furthermore, that addition of McN-3716 (Figure 4) to isolated diaphragms produced an equivalent activation of the oxidation of both glucose- $1\text{-}^{14}\text{C}$  and glucose- $6\text{-}^{14}\text{C}$  is consistent with a stimulation of glycolysis rather than of the hexose monophosphate shunt. Also, as discussed by Fritz and Kaplan,<sup>21</sup> there is little evidence for the operation of the hexose monophosphate shunt in rat diaphragm. Additional support for the above hypothesis has also come from experiments using isolated rat hepatocytes.<sup>16</sup> McN-3802 reversed the inhibition of pyruvate dehydrogenase that occurred on addition of oleic acid, presumably by lowering the concentrations of acetyl CoA or by lowering the NADH/NAD ratio.

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