Journal of Cardiovascular Magnetic Resonance®, 3(1), 35–45 (2001)

^{1}H-MRS Detected Lipolysis in Diabetic Rat Hearts Requires Neutral Lipase*

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ABSTRACT

Purpose: Triacylglycerol (TAG) lipolysis increases in diabetic hearts. However, it is not known which pathway for lipolysis catalyzes this process. Thus, using ^{1}H-magnetic resonance spectroscopy (MRS), we determined whether TAG lipolysis in diabetic rat hearts requires acid lipase or neutral lipase activity. Methods: Rats were given IP injections of 110 mg streptozotocin (STZ)/kg. Forty-eight to 72 h after this treatment, all rats exhibited ketotic diabetes. The hearts of these ketotic rats were isolated, perfused isovolumically, and analyzed using ^{1}H-MRS. Results: The content of methylene protons $(C\mathbf{H}_2)_n-$ and other fatty acid protons, measured using ^{1}H-MRS, increased in hearts isolated from STZ-treated compared to untreated rats. This increase in heart $-(C\mathbf{H}_2)_n-$ was directly related to the chemical content of heart TAGs. If isolated diabetic hearts were perfused with either glucose or glucose plus the acid lipase inhibitor methylamine, then heart content of TAG, measured as $(C\mathbf{H}_2)_n$, decreased at rates of approximately 130 nmol TAG/gdw/min throughout a 55-min perfusion. If diabetic hearts were pretreated with the neutral lipase inhibitor diethyl-p-nitro-phenylphosphate (DNPP) and perfused with glucose, then heart TAG content, measured as $(C\mathbf{H}_2)_n$, did not change during perfusion. Conclusions: ^{1}H-MRS can detect the TAG and the net lipolysis of TAG in diabetic rat hearts. Net TAG lipolysis in diabetic rat hearts requires neutral lipase.

KEY WORDS: Diabetes; ^{1}H-MRS; Lipolysis; Neutral lipase; Triacylglycerols

*Review under the Aegis of Invited Editor Stefan Neubauer.

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INTRODUCTION

Heart content of triacylglycerols increases significantly in ketotic diabetes (1). These neutral lipids, localized either to membrane-bound cytoplasmic inclusions or to lysosomes (2), serve as an endogenous pool of fatty acids that can contribute significantly to heart energy metabolism (3).

Both the steady-state content of triacylglycerols in the heart and the turnover of this pool of lipids reflect the intracellular rates of free fatty acid esterification into triacylglycerols and the intracellular rate of triacylglycerol lipolysis (2). Two distinct pathways for lipolysis exist in the heart. The first pathway is present in lysosomes and an acid lipase activity regulates flux through this pathway (4). Lysosomotrophic agents, such as methylamine and chloroquine, inhibit acid lipase-dependent lipolysis (4). The second pathway for lipolysis is associated with the cytoplasmic triacylglycerol-rich inclusions themselves (2). A hormone-sensitive neutral lipase regulates flux through this pathway (5). DNPP inhibits irreversibly the neutral lipase and, thereby, neutral lipase-dependent lipolysis (6,7).

The acid lipase pathway is the main pathway for triacylglycerol lipolysis in the normal rat heart (4). In contrast, the neutral lipase pathway is the predominant pathway for lipolysis in hypertriglyceridemic hearts, for example, in hearts isolated from rats fed rapeseed oil (RSO) (4). The contribution of the neutral lipase and the acid lipase pathways to lipolysis in the hypertriglyceridemic hearts of severely diabetic rats, however, remains unresolved.

Most investigations of triacylglycerol metabolism in diabetic hearts have used radiochemical or biochemical techniques. For example, since heart muscle contains little glycerol kinase (8), release of glycerol either from perfused hearts or from myocytes can provide one measure of cardiac triacylglycerol lipolysis (6). Likewise, triacylglycerol lipolysis can be measured by prelabeling the heart triacylglycerol pool with ^{14}C-labeled fatty acids (9). While providing key pieces of information about triacylglycerol lipolysis in the heart, these approaches do not provide a direct, continual assessment of the net triacylglycerol content of the heart. ^{1}H- and ^{13}C-MRS can provide such information by quantitating the triacylglycerol content, rates of net triacylglycerol lipolysis, and rates of fatty acid esterification into triacylglycerols in normal hearts or in hearts isolated from rats fed RSO (10,11,12).

Using ^{1}H-MRS methodology, therefore, we investigated whether net lipolysis in diabetic hearts requires acid lipase activity, neutral lipase activity, or both. To answer this question, experiments were performed that establish for the first time that ^{1}H-MRS can quantitate the triacylglycerol content and measure the rate of net triacylglycerol lipolysis in hearts isolated from diabetic rats. Furthermore, we demonstrate for the first time that net lipolysis in diabetic heats requires only neutral lipase activity.

METHODS

These investigations conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, Revised 1985), as enforced by the Institutional Animal Use and Care Central Committee of the University of Alabama at Birmingham.

Induction of Diabetes

Streptozotocin (STZ) was suspended in 0.5 M Na^{+} citrate (pH 5.4) at a concentration of 70 mg/mL. Male Sprague Dawley rats ($n = 30$ total) were given a single IP injection of 110 mg STZ/kg (13). Twenty-four to 72 h following treatment with STZ, one group of rats ($n = 10$) was sacrificed and used to measure the relationship between the chemical content of heart triacylglycerol and the heart content of ^{1}H-MRS observable fatty acid protons. The remaining rats ($n = 20$) were recovered 72 h following treatment with STZ, and the glucose and ketone body content of their urine was assessed using commercially available diagnostic chemstrips. All of these rats were ketonuric. Their hearts were used to measure rates of net triacylglycerol lipolysis.

Chemical Measurement of the Heart Content of Triacylglycerol

Hearts were isolated either from rats treated with STZ ($n = 10$, total) for 24, 48, or 72 h, or from rats treated with citrate vehicle ($n = 2$). During this range of times following STZ treatment, a progressive increase has been reported to occur in the triacylglycerol content of the heart (13). These hearts were perfused in a retrograde fashion (11) with glucose and acetate as exogenous substrates. ^{1}H-MRS spectra of these hearts were then acquired and analyzed for their content of $(C\boldsymbol{H}_2)_n$ protons.

At the end of these perfusions, each heart was snap-frozen in liquid nitrogen and stored at -20°C. Frozen

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hearts were pulverized, and the total lipids from 200 to 400 mg of heart powder were extracted and concentrated under nitrogen. The triacylglycerols in these samples were selectively saponified using tetraethyl ammonium hydroxide and the glycerol content of these saponified samples was assayed (10).

Preparation of Isolated Hearts and Perfusion Protocols

Twenty Ketotic diabetic rats were given a single IP injection of 300 units heparin/kg. Ten to 15 min later, all rats were given a single IP injection of 0.2 g pentobarbital/kg. Under anesthesia, the hearts were removed from these rats, chilled in ice-cold Krebs-Henseliet (KH) buffer (19), their aortas cannulated, and their left ventricles vented at the apex. Retrograde perfusion was initiated using KH containing 1.75 m*M* $CaCl_2$, with 10 m*M* glucose and 7.5 m*M* Na^+ acetate as exogenous substrates. Acetate was used to suppress lipolysis during the stabilization period prior to the initiation of any specific experimental protocol (10). All perfusates were aerated with 95% O_2/5% CO_2. All perfusions were carried out at 37°C, a perfusate pH of 7.4, and a perfusion pressure of 80 mm Hg.

A latex balloon was placed in the left ventricle of all perfused hearts and sutured in place. This intraventricular balloon was connected via a fluid-filled catheter to a pressure transducer. The balloon transducer assembly was used to measure heart rate, LVEDP, and LVDP. A Keithley System 570 analog-to-digital converter (Keithley Metrabyte, Taunton, MA) was used to digitize pressure traces, and these were stored on a personal computer for subsequent analysis. At the outset of all perfusions, the volume of the balloon was adjusted so that the LVEDP of each heart reached approximately 9 mm Hg. A 4-mm glass sphere containing 25 μl of a 180-m*M* solution of 3-(trimethylsilyl) tetradeutero sodium propionate (TSP), a ^{1}H standard, then was positioned next to all hearts and attached to the perfusion cannula. All hearts were then placed into a 20-mm MRS tube and inserted into the bore of a Bruker AM 360 NMR spectrometer (Bruker Instrument Co., Billerica, MA). Hearts were allowed to stabilize for 15 to 20 min, during which time the probe was tuned and the samples were shimmed.

Four perfusion conditions were studied using hearts isolated from the 20 ketotic diabetic rats. One group of five diabetic hearts was perfused with KH containing 10 m*M* glucose and 7.5 m*M* Na^+ acetate during the stabilization period. Following the acquisition of three baseline ^{1}H-MRS spectra, these hearts were then perfused during the experimental period with 10 m*M* glucose as sole exogenous substrate. A second group of five diabetic hearts was perfused with KH containing 10 m*M* glucose and 7.5 m*M* Na^+ acetate during both the stabilization and the experimental periods. A third group of five diabetic hearts was perfused with KH containing 10 m*M* glucose, 7.5 m*M* Na^+ acetate, and 5 m*M* methylamine during the stabilization period. These hearts were then perfused with KH containing 10 m*M* glucose and 5 m*M* methylamine during the experimental period. The fourth and final group of five diabetic hearts was perfused with KH containing 10 m*M* glucose, 7.5 m*M* Na^+ acetate, and 0.04 m*M* DNPP during the stabilization period, followed by perfusion with KH containing 10 m*M* glucose during the experimental period. ^{1}H-MRS spectra were acquired throughout all perfusions in these four groups.

Hearts in the first three experimental groups were allowed to beat at their natural rates. Since DNPP induces bradycardia (14), hearts in the last experimental group were paced at 250 beats per min.

The heart rate and the left ventricular developed pressure (LVDP) for each heart were multiplied to give heart rate pressure product (RPP). RPP is reported as a measure of heart mechanical performance.

^{1}H-MRS of Perfused Hearts

^{1}H-MRS spectra were acquired using the decoupling coil of a 20 mm ^{31}P-MRS probe. ^{1}H-MRS FIDs had a sweep width of 5000 Hz, 8192 data points, and 64 averages per spectrum. Water suppressed excitation was achieved using the 1331 pulse sequence with an interpulse delay of 400 μs, the transmitter frequency set at the water resonance, and a 5-s delay between acquisitions (10). Compensation for the frequency dependent variation in transverse magnetization of the 1331 sequence was made using product operators.

The NMR1 software package (Tripos, East Syracuse, NY) was used to process all ^{1}H-MRS spectra. All ^{1}H FIDs were processed using a line broadening of 3 Hz. The external TSP standard was used to quantify both the content of and the changes in the areas of ^{1}H-MRS resonances during perfusion.

Statistics

All values are reported as means ± standard error. Comparisons between groups were made using the unpaired

Student's *t* test (two tailed), and a Bonferroni correction for multiple comparisons. Comparison within groups used repeated measures ANOVA. A probability value of 0.05 or less was considered statistically significant.

Materials

Streptozotocin and DNPP were from Sigma Chemical Co. (St. Louis, MO). Chemstrips for analysis of glucosuria and ketonuria were obtained from Boehringer Mannheim (Indianapolis, IN). All other chemicals were of standard laboratory grade.

RESULTS

Experiments were performed to determine whether the hypertriglyceridemia that occurs in ketotic diabetic rat hearts (1) affects the intensity of their ^{1}H-MRS observable fatty acid resonances. Rats received either a single IP injection of 110 mg STZ/kg or citrate vehicle. As expected (1), rats became severely ketonuric 72 h following treatment with STZ. Hearts were isolated from both groups of rats, perfused isovolumically, and analyzed using ^{1}H-MRS (10). The ^{1}H-MRS peak area of resonances assigned to fatty acid protons increased markedly in hearts from ketonuric rats when compared to hearts isolated from rats treated with citrate alone (Fig. 1, cp. spectra A and B). Resonances corresponding to C$\boldsymbol{H}_3$ protons (0.86 ppm relative to TSP at 0 ppm), $-(C\boldsymbol{H}_2)_n-$ protons (1.26 ppm), γ ($-C\boldsymbol{H}_2-$) protons (1.55 ppm), $-C\boldsymbol{H}_2-CH=$ protons (1.97 ppm), and $\beta(-C\boldsymbol{H}_2-)$ protons (2.19 ppm) all increased in the hearts of ketonuric rats.

Experiments were performed to establish the relationship between this increased heart content of $-(C\boldsymbol{H}_2)_n-$ in ketonuric rats and the increased heart content of

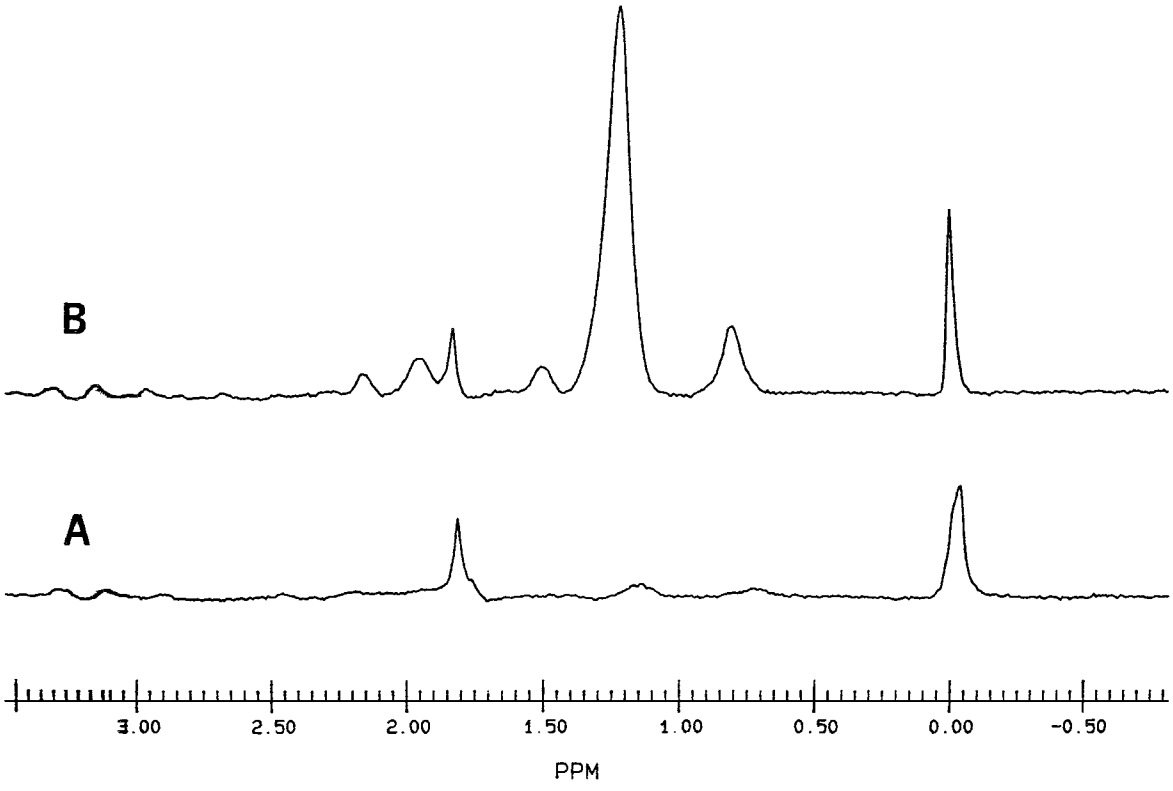

Figure 1. ^{1}H-MRS spectroscopy shows that the content of fatty acid protons increases in hearts isolated from ketonuric rats when compared to control hearts. Ketotic diabetes was induced in rats and their hearts were isolated and perfused isovolumically as described in Materials and Methods. ^{1}H-MRS spectra were acquired as described in Materials and Methods. Spectrum A was acquired from an untreated rat heart. Spectrum B was acquired from hearts isolated from rats experiencing ketotic diabetes. The rightmost resonance is TSP (0.0 ppm). Other resonances are assigned as β $-(C\boldsymbol{H}_2)-$, 2.19 ppm; $-C\boldsymbol{H}_2-CH=$, 1.97 ppm; Na^+ acetate, 1.86 ppm; γ $-(C\boldsymbol{H}_2)$, 1.55 ppm; $-(C\boldsymbol{H}_2)_n-$, 1.26 ppm; $-(C\boldsymbol{H}_3)$, 0.86 ppm.

triacylglycerols that occurs in ketotic diabetes (1). Ten rats were treated with STZ and 2 rats were treated with citrate alone. Twenty-four to 72 h following their treatment with STZ or citrate, rats were sacrificed and their hearts were perfused isovolumically. ^{1}H-MRS spectra were acquired from these hearts, and these spectra were analyzed for the intensity of their $-(CH_2)_n-$ resonance. On average, there are 64.3 mol of $-(CH_2)_n-$ per mol of triacylglycerols in diabetic hearts (15). This factor was used to convert a heart content of $-(CH_2)_n-$ to a heart content of triacylglycerols using ^{1}H-MRS approaches that have been described previously (10). The total lipids were extracted from these hearts, and the chemical content of triacylglycerols was measured in these extracts. Comparing the ^{1}H-MRS measurements of heart triacylglycerols with the chemical measurements of the actual triacylglycerol content of ketonuric hearts gave a direct linear relationship (Fig. 2, $m = 0.93$, $r = 0.92$).

Two sets of experiments were performed to establish whether the ^{1}H-MRS observable triacylglycerols in ketonuric rat hearts were metabolically active. First, hearts ($n = 5$) isolated from ketonuric rats were perfused with glucose as the sole exogenous substrate during the experimental period. A time-dependent decrease occurred in the peak area of the $-(CH_2)_n-$ resonance of these hearts during the course of these perfusions (Figs. 3A and 4). Using the linear relationship between the heart content of triacylglycerols measured using ^{1}H-MRS and the heart content of triacylglycerols measured chemically (Fig. 2), it was estimated that hearts isolated from ketonuric rats lipolyzed 135.6 ± 8.2 nmol triacylglycerol per gram dry wt-min when perfused with glucose as sole exogenous substrate.

Second, hearts ($n = 5$) isolated from ketonuric rats were perfused with glucose and acetate during both the stabilization and the experimental periods. Acetate is an alternate metabolic substrate that suppresses lipolysis (1,10). The peak area of heart $-(CH_2)_n-$ decreased slowly throughout the course of these perfusions (Fig. 4). It was estimated that hearts isolated from ketonuric rats lipolyzed 21.0 ± 5.6 nmol of triacylglycerol per gram dry wt-min when perfused with glucose and acetate as exogenous substrates. These changes in heart $-(CH_2)_n-$, a direct measure of heart triacylglycerol content, were linear (Fig. 4) and were significantly different from each other ($p < 0.05$).

Two sets of experiments were performed to test whether the acid lipase or the neutral lipase catalyzed triacylglycerol lipolysis in hearts from ketonuric rats. First, hearts ($n = 5$) isolated from ketonuric rats were perfused with glucose and methylamine during the

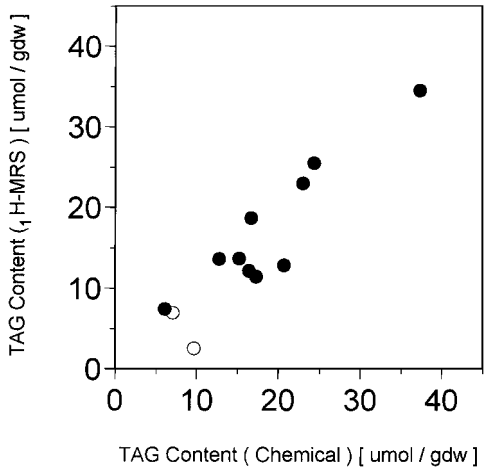

Figure 2. The ^{1}H-MRS peak area of $(CH_2)_n$ in the hearts of ketotic rats correlates with their chemical content of triacylglycerols. The content of $-(CH_2)_n-$ protons in hearts of untreated rats (○) and hearts from STZ-treated rats (●) was measured using ^{1}H-MRS as described in Materials and Methods. ^{1}H-MRS $(CH_2)_n$ resonance peak area was converted to a ^{1}H-MRS measured triacylglycerol content (left axis) as described in Methods. The chemical content of triacylglycerol in these hearts was determined as described in Methods. The data from these two measurements fit a line with r equal to 0.92, and a slope of 0.93.

experimental period. Methylamine is an inhibitor of acid lipase activity (4). A time-dependent decrease occurred in the $-(CH_2)_n-$ peak area of ketonuric hearts throughout the course of perfusion with glucose and methylamine (Figs. 3B and 4). It was estimated that ketonuric hearts lipolyzed 129.4 ± 11.1 nmol triacylglycerol per gram dry wt-min during perfusion with glucose and methylamine. The rate of $(CH_2)_n$ decrease in hearts perfused with glucose and methylamine was not different from rates measured in hearts perfused with glucose alone.

Second, hearts ($n = 5$) from ketonuric rats were perfused with glucose, acetate, and DNPP during the stabilization period, and then with glucose alone during the experimental period. DNPP is a potent inhibitor of esterases such as neutral lipase (7,14). The $-(CH_2)_n-$ peak area of glucose-perfused, ketonuric hearts that had been pretreated with DNPP decreased much less rapidly compared to ketonuric hearts perfused with glucose and methylamine (cp. Figs. 3B, 3C, and 4). It was estimated that hearts isolated from ketonuric rats and pretreated with 0.04 m*M* DNPP lipolyzed 2.5 ± 6.6 nmol triacylglycerol per gram dry wt-min when perfused with glucose as sole exogenous substrate. The rate of $(CH_2)_n$ decrease in these DNPP-treated, glucose-perfused hearts was significantly

different from the rate measured for hearts perfused with glucose and methylamine ($p < 0.05$).

The mechanical performance of these four groups of diabetic hearts was measured throughout perfusion. Following 100 total min of perfusion, that is, at the end of the stabilization and the experimental periods, the RPP of hearts perfused with glucose was 18.6 ± 1.8 kmm Hg/min, while the RPP of hearts perfused with glucose and acetate was 19.7 ± 2.9 kmm Hg/min. These values were not statistically different. The RPP for hearts perfused with glucose and methylamine was 18.7 ± 1.8 kmm Hg/min, while it was 18.1 ± 2.1 kmm Hg/min for hearts perfused with glucose and DNPP. These values were not statistically different. In addition, these values were not significantly different from the RPPs measured in the first 20 min of perfusion (data not shown). Thus, all preparations of hearts were stable, in keeping with previous reports (13).

DISCUSSION

Ketotic diabetes increases significantly the heart content of triacylglycerols (1). Data presented in this paper demonstrate for the first time that ^{1}H-MRS can detect these increases in heart triacylglycerols, and that these increases in the intensity of ^{1}H-MRS fatty acid proton resonances track directly with heart triacylglycerol content. This result is similar to those reported for hearts isolated from rats fed a diet high in RSO (10,11). Thus, these results demonstrate the general utility of ^{1}H-MRS in measuring

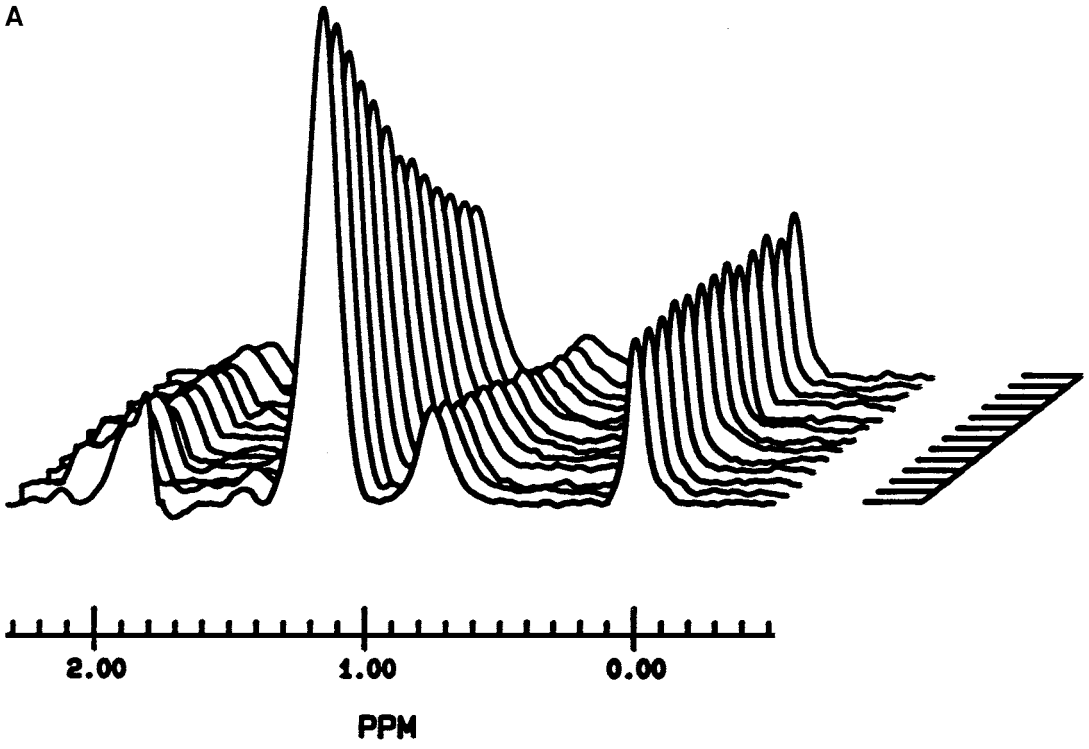

Figure 3. DNPP, an inhibitor of neutral lipase, prevents the decrease in $(CH_2)_n$ that occurs in glucose-perfused diabetic hearts, while methylamine, an inhibitor of lysosomal acid lipase, does not. Diabetes was induced in rats and their hearts were isolated and perfused as described in Methods. ^{1}H-MRS spectra were acquired as described in Methods. Figure 3A shows ^{1}H-MRS spectra typical of those obtained from hearts perfused with KH containing 10 m*M* glucose and 7.5 m*M* Na^+ acetate as exogenous substrates during the first spectrum, and then perfused with KH containing 10 m*M* glucose alone. Figure 3B shows ^{1}H-MRS spectra typical of those obtained from hearts perfused with KH containing 10 m*M* glucose and 7.5 m*M* Na^+ acetate for the first spectrum, and then perfused with KH containing glucose and 5 m*M* methylamine. Figure 3C shows ^{1}H-MRS spectra typical of those obtained from hearts perfused with KH containing 10 m*M* glucose, 7.5 m*M* Na^+ acetate, and 0.04 m*M* DNPP for the first spectrum. This heart was then perfused with KH containing glucose. The rightmost resonance in all panels is the TSP standard (0.0 ppm). Each spectrum took approximately eight minutes to acquire.

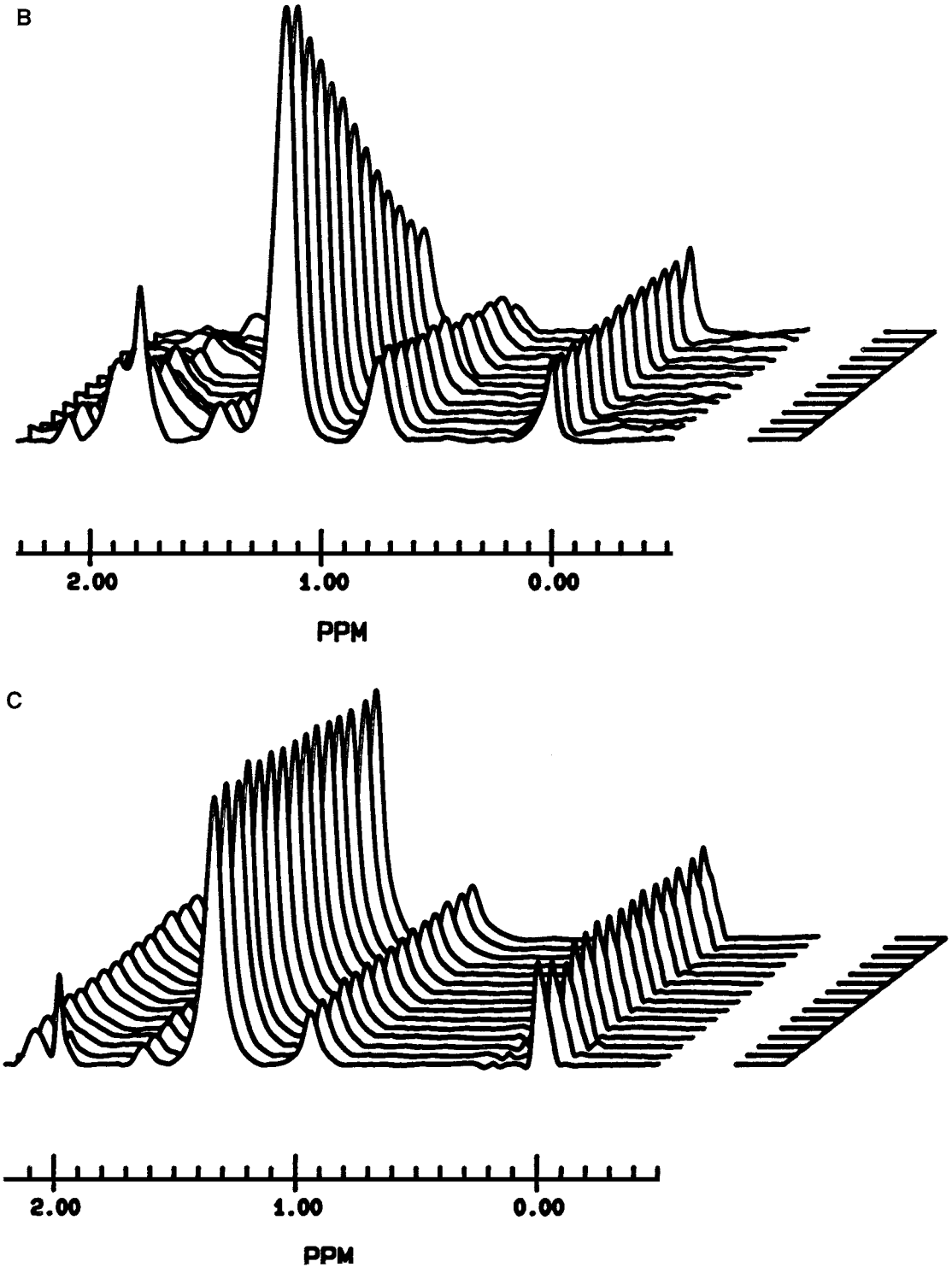

Figure 3. *Continued*

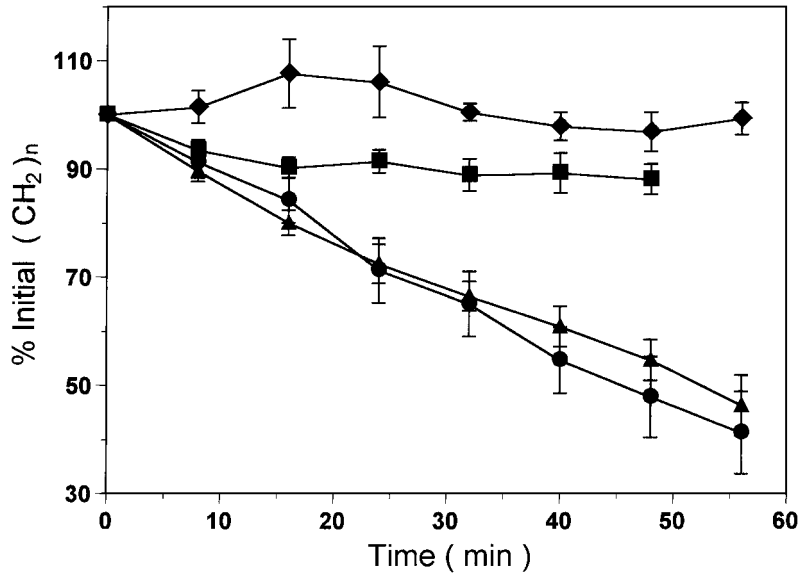

Figure 4. Decreases in the $-(CH_2)_n-$ content of perfused diabetic rat hearts under the four groups of experimental conditions. Diabetes was induced in rats and their hearts were isolated and perfused as per Methods. ^{1}H-MRS spectra were acquired as per Methods. Hearts were perfused with KH containing either (i) 10 mM glucose (●, $n = 5$), (ii) 10 mM glucose and 7.5 mM Na^+ acetate (■, $n = 5$), (iii) 10 mM glucose and 5 mM methylamine (▲, $n = 5$), or (iv) were pre-treated with 0.04 mM DNPP and then perfused with 10 mM glucose (◆, $n = 5$) during the experimental period. Values are the mean ± the S.E.M. of the percent decrease in heart content of $(CH_2)_n$ compared to the initial content of $(CH_2)_n$ in that heart.

the rates of net triacylglycerol lipolysis in hypertriglyceridemic hearts. ^{1}H-MRS may also find utility in tracking net increases in heart triacylglycerol under conditions known to increase the intracellular content of this lipid, for example in ischemic hearts (16).

Lipolysis of heart triacylglycerols occurs via two distinct pathways. The first pathway is associated with cardiac lysosomes and requires an acid lipase activity. Lysosomotrophic agents, such as methylamine and chloroquine, inhibit its activity (2,4). The second pathway for lipolysis is associated with the cytosolic triacylglycerol droplets themselves and requires a hormone-sensitive neutral lipase activity (2,5). Esterase inhibitors, such as DNPP, inhibit neutral lipase (4,6,7). While the activities of the acid and the neutral lipases have been measured in severely diabetic hearts (17), their relative contribution to net triacylglycerol lipolysis in the diabetic heart remains unresolved. Our results support the conclusion that triacylglycerol lipolysis in diabetic hearts requires only neutral lipase activity and not acid lipase activity.

Normally, lysosomes play a critical role in myocardial lipolysis (2). For example, despite the presence of roughly equal levels of neutral lipase and acid lipase activities in normal hearts, approximately 90% of lipolysis in normal hearts is sensitive to inhibitors of acid lipase (4). This result indicates that triacylglycerols associated with lysosomes may be the metabolically active pool in normal hearts. The relative contribution of the acid and the neutral lipases to flux through cardiac lipolysis, however, is different in rats fed RSO so as to induce myocardial hypertriglyceridemia. Here, acid lipase activity accounts for roughly 30% of total lipase activity, and approximately 30% of total lipolysis (4).

It is clear both from our results (Fig. 4) and from preceding reports (18) that in diabetic hearts, despite the presence of significant levels of acid lipase activity (17), this enzyme is not involved in triacylglycerol lipolysis. It may be that the partitioning of triacylglycerols between the pool present in lysosomes and the pool present in cytoplasmic droplets is markedly altered in the hearts of diabetic rats. If low levels of triacylglycerols were associated with lysosomes in diabetic hearts, then lipolysis in the diabetic heart would be relatively insensitive to inhibitors of acid lipase. Further studies are required to establish whether a decrease occurs in the amount of myocardial triacylglycerols associated with lysosomes in diabetic hearts.

It has been proposed that the basal rates of triacylglycerol lipolysis in diabetic hearts increase solely because of increases in the intracellular levels of triacylglycerol (19,20). This conclusion, however, does not account for several facts. For example, while similar rates of lipolysis occur in hearts isolated either from rats fed RSO or from diabetic rats, neutral lipase catalyzes 60–70% of overall lipolysis in the former model (14), and 100% in the latter model (Fig. 4) (18). Thus, lipolysis in hearts isolated from rats fed RSO originates from two physically distinct pools of triacylglycerols, one associated with lysosomes and a second associated with cytoplasmic lipid droplets. Acid lipase and neutral lipase regulate lipolysis from these two pools, respectively (2,4). It is not clear how lipolysis in RSO hearts could occur at rates similar to those measured in diabetic hearts, where lipolysis occurs solely from the cytoplasmic droplets and is regulated solely by neutral lipase (Fig. 4) (2,18). In addition, five-fold lower levels of neutral lipase activity have been reported in diabetic hearts compared to hearts isolated from rats fed RSO, at similar absolute levels of triacylglycerol (17,19). Nonetheless, the maximum rates of lipolysis are similar in these two models of the hypertriglyceridemic heart (17,20). Consequently, regulation of the activity of neutral lipase itself probably plays a key role in setting the rates of net triacylglycerol lipolysis in these hearts.

Phosphorylation of neutral lipase at two neighboring serine residues regulates both its catalytic activity (5) and its intracellular location (21). Cyclic AMP- and cyclic GMP-dependent protein kinases phosphorylate neutral lipase at a "regulatory" site, serine 563 in the rat (5), thereby activating lipase catalytic activity (5) and inducing its translocation onto cytoplasmic triacylglycerol droplets (21). This translocation reversibly associates neutral lipase with its substrate. Neutral lipase also contains a "basal" phosphorylation site, serine 565 in the rat (5). AMP-activated protein kinase (AMPK) and calmodulin-dependent protein kinase phosphorylate this site and prevent cyclic nucleotide-dependent protein kinase activation of neutral lipase (5). Phosphorylating either of these two sites hinders the subsequent phosphorylation of the remaining site (5). Consequently, the net activity of neutral lipase represents a balance between the degree of phosphorylation of its regulatory site and its basal site.

Treating rats with STZ markedly decreases their serum insulin (22–24). This change could affect the activity of neutral lipase in the diabetic heart in two ways. First, low levels of serum insulin increase heart cyclic AMP (25,26). This change may activate cyclic AMP-dependent protein kinase that would increase the phosphorylation of the regulatory site of neutral lipase and, thereby, increase neutral lipase activity. Second, insulin depresses AMPK activity in myocytes (27,28). In ketotic diabetic rats, serum insulin is low, therefore AMPK activity may increase (29). This increase could enhance phosphorylation of the basal site on neutral lipase (5) and lower neutral lipase activity. Measuring the phosphorylation state, activity, and intracellular location of neutral lipase in diabetic hearts, and the activity of AMPK in diabetic hearts perfused with glucose, would establish whether activation of neutral lipase in diabetic hearts is responsible for the enhanced rates of lipolysis in these hearts.

In conclusion, ^{1}H-MRS demonstrates that neutral lipase catalyzes triacylglycerol lipolysis in diabetic hearts. Elevated rates of lipolysis occur in glucose-perfused hearts isolated from diabetic rats, and the mechanism for this activation of neutral lipase-catalyzed lipolysis remains unresolved. Either increased phosphorylation of the neutral lipase regulatory site or decreased phosphorylation of its basal site may enhance lipolysis in diabetic hearts.

ABBREVIATIONS

(CH_3)	triacylglycerol methyl protons
$(CH_2)_n$	triacylglycerol methylene protons
DNPP	diethyl-*p*-nitrophenylphosphate
FIDs	free induction decays
IP	intra-peritoneal
KH	Krebs-Henseliet
LVDP	total left ventricular developed pressure
LVEDP	left ventricular end diastolic pressure
MRS	magnetic resonance spectroscopy
RPP	rate pressure product
RSO	rapeseed oil
STZ	streptozotocin
TSP	3-(trimethylsilyl) tetradeutero sodium propionate

ACKNOWLEDGMENTS

Grants HL 52977 (P.E.W.), HL 57965 (G.M.P.), and HL 46033 (J.A.B.) supported this work.

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Received March 1, 2000
Accepted July 11, 2000

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