EFFECTS OF DIABETES ON COMPOSITION AND METABOLISM OF HEART LIPIDS

by

DENNIS J. PAULSON, B.S., M.A.

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CHAPTER I

GENERAL INTRODUCTION AND LITERATURE REVIEW

The overall objective of this study was to examine possible alterations in the composition and metabolism of lipids by the diabetic heart. Two sets of experiments were designed to investigate this problem. The first study, Part I, was a descriptive examination of the lipid composition of normal and 12-day streptozotocin-induced diabetic rat hearts. Changes in content and fatty acid composition of myocardial triacylglycerols (TG), triacylglycerol fatty acids (TGFA) and tissue free fatty acids (tFFA) were determined, along with serum free fatty acid (sFFA) concentration and composition. Heart phospholipid (PL) content was measured also. The second set of experiments, Part II, was an examination of the dynamics of lipid metabolism in normal and diabetic perfused rat hearts. TG lipolysis, synthesis and oxidation were measured in hearts perfused under conditions that simulated the in vivo environment of the heart.

This chapter is devoted to a review of the literature on lipid (particularly TG) metabolism in the normal and diabetic myocardium. In addition, possible detrimental

effects of abnormal lipid metabolism on the normal and diabetic heart are discussed.

Lipid Composition of the Normal Heart

Lipids of the rat heart make up about 14 percent of the total dry weight (69). Of the total lipids, 85 percent is made up of PL and the other 15 percent is accounted for by neutral lipids plus free fatty acids. TG comprises most of the neutral lipids with only small amounts of mono (MG) and diacylglycerol (DG), tFFA, cholesterol (C), and cholesterol esters (CE) being present. There are normally about 30 to 45 μmoles of fatty acid per gram dry weight in the heart as TGFA (12, 19, 20, 23, 27, 65, 69, 83), whereas the tFFA content ranges from 1.5 to 4.0 μmoles per gram dry weight (69).

Lipid Metabolism in the Normal Heart

In the normal heart, TGFA serve as an important energy reserve (13, 19, 65, 69, 83), whereas PL are primarily structural lipids. A schematic of normal heart lipid metabolism is shown in Figure 1. The rate limiting enzyme in TG synthesis is thought to be α-glycerol phosphate acyltransferase (56). This enzyme catalyzes the formation of phosphatidic acid from α-glycerol phosphate and long chain acyl CoA. Reportedly, the enzyme has a lower Km for

Lipid Metabolism In The Normal Heart

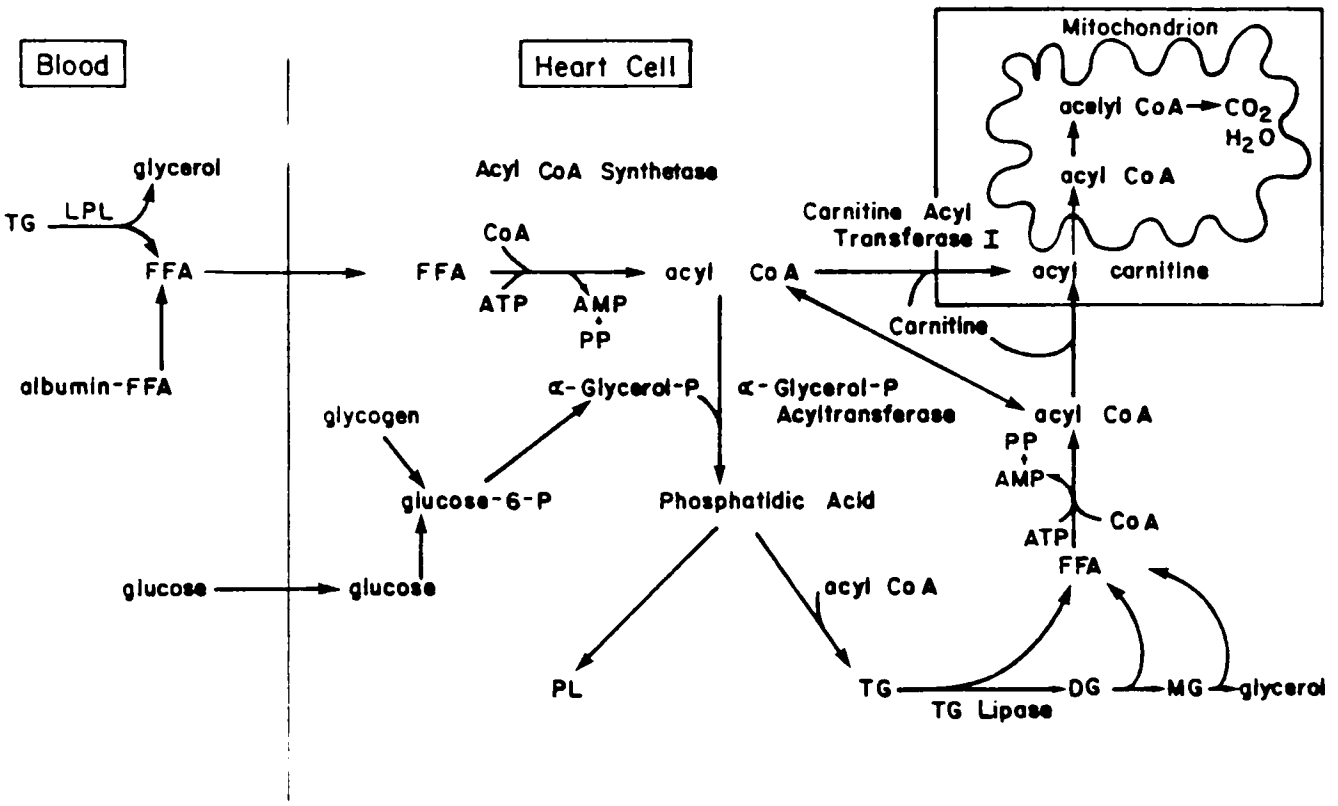

Figure 1: A schematic of normal heart lipid metabolism. Abbreviations: TG = Triacylglycerol, DG = Diacylglycerol, MG = Monoacylglycerol, tFFA = Tissue Free Fatty Acids, LPL = Lipoprotein Lipase.

acyl CoA than does carnitine acyl transferase (8). The formation of α-glycerol phosphate from glucose or glycogen (23, 96) is considered to be the limiting reactant in complex lipid biosynthesis. Acyl CoA diverted into tissue lipids are preferentially incorporated into TGFA (12, 15, 23, 65, 68, 83). In the presence of other oxidizable substrates, such as ketone bodies, esterification of exogenous FFA to TG is enhanced (68).

Carbon chain length and degree of unsaturation of exogenous FFA also affect their uptake and metabolic fates, principally oxidation and incorporation into tissue lipids. As chain length of saturated fatty acids with an even number of carbon atoms increases, uptake and oxidation decrease while incorporation into tissue lipids has been shown to increase. Uptake of oleic acid is greater than that of stearic or linoleic acid (23). Oleic acid is incorporated preferentially into TG, whereas erucic and stearic acid accumulate as tissue FFA (91). Linoleic and linolenic acid are found primarily in the phospholipid fraction. Palmitate is found chiefly as the carnitine derivative. There is also a positional specificity for the esterification of fatty acids. Palmitate is preferentially incorporated into the 1-position, while oleate preferentially inserts at the 2-position (56).

Studies with adipose tissue have suggested that the major control site of TG hydrolysis is at the level of TG lipase. By inference, TG lipase is also believed to be the controlling enzyme in heart TG lipolysis (5, 13, 65, 83). Studies on lipolysis are technically difficult since the heart possesses many lipases, i.e., TG lipase (5, 13, 65, 83), lipoprotein lipase (1, 49, 53), DG lipase (5) and MG lipase (5). To my knowledge, there is only one study that compares TG, DG and MG lipase activity in the heart. Biale et al. (5) showed that, in whole heart homogenates, rates of cleavage of DG and MG were much faster than that of TG, thus suggesting that TG lipase is rate-limiting in TG lipolysis.

The regulation of TG metabolism is very complex and not well understood. There appear to be multiple pools of TG in the heart. Some pools may be rapidly mobilized by TG lipase while other pools are more slowly metabolized (13, 19, 65, 83, 88). TG lipase of the heart is similar to adipose tissue TG lipase in that both epinephrine and glucagon activate lipolysis; presumably acting through a cyclic AMP mechanism (13, 17, 55, 65, 83). However, there is very little solid evidence to implicate the cyclic AMP system in myocardial lipolysis (13, 55). Heart TG lipase also differs from adipose TG lipase in that insulin does

not show an antilipolytic effect (55). An important type of regulation of TG lipolysis involves exogenous FFA in high concentrations. TG lipolysis is inhibited when exogenous FFA are elevated (12). This feedback mechanism has been shown to override epinephrine-induced stimulation of lipolysis (17). In addition, ketone bodies are reported to inhibit isoproterenol-stimulated lipolysis (45). Increased ventricular pressure development also stimulates oxidation of TGFA (16). Endogenous TG are utilized for energy production in preference to exogenous glucose (13). However, there are also reports that glucose has a sparing effect on TG utilization (19, 31, 64). It is unknown whether TG lipase activity is affected by the TGFA composition, i.e., substrate acyl group specificity.

The FFA produced from TG lipolysis as well as exogenous uptake are activated to form long chain acyl CoA by the enzyme acyl CoA synthetase (8, 65). The reaction is controlled by the availability of substrates (tFFA and CoA) and inhibition due to product accumulation (long chain acyl CoA and AMP) (44, 65). Long chain acyl CoA penetrates the inner mitochondrial membrane only in the presence of carnitine (44, 65). Acyl carnitine is formed outside mitochondria by the enzyme carnitine acyltransferase I (8, 44, 65). The acyl carnitine is transported across the inner mitochondrial membrane in exchange for carnitine. On the

inner side of the mitochondrial membrane, the acyl carnitine is reconverted to carnitine and acyl CoA by the carnitine acyltransferase II. The acyl CoA is further metabolized by β-oxidation in which two carbon units as acetyl CoA are removed from the carboxyl end of the fatty acid with every turn of the β-oxidation cycle. Acetyl CoA then enters the Krebs cycle for further oxidation and energy production (44, 65). Short chain fatty acids are also activated to acyl CoA, but transport of these fatty acids into the mitochondria is not dependent upon the carnitine acyl transferase system (44, 65).

Lipid Composition of the Diabetic Heart

In the diabetic heart, the intracellular content of TG (20, 63, 76, 77, 78), tFFA (28, 30), long chain acyl CoA (6, 24, 30) and acyl carnitine (6, 24, 30) have been shown to be increased while the PL content remained unchanged (20, 78). However, one report has shown a significant reduction in PL content in the alloxan diabetic rat heart (11). The decrease was restricted to phosphatidylethanolamine and lysophosphatidylcholine. Other lipid abnormalities found in diabetes were: a) decreased carnitine content (6, 24), b) increased body turnover of carnitine (59), c) increased cardiac CoA content (24), and d) increased blood concentration of FFA and TG (80).

Lipid Metabolism in the Diabetic Myocardium

The mechanisms underlying the alterations in lipid metabolism in the diabetic heart are not fully understood. The increase in heart TG in the 48-hour alloxan-diabetic rat required growth hormone and cortisol and occurred when the rat consumed a fat-free diet (20). Insulin treatment, *in vivo*, prevented the increase in heart TG (63, 78). Collectively, these studies suggested that increased deposition of myocardial TGFA was derived from enhanced mobilization of adipose tissue fatty acids.

Accumulation of TG in the heart could result from increased synthesis, decreased mobilization or both. Several studies have suggested that TG synthesis in the diabetic myocardium was greatly enhanced. The incorporation of radioactively labeled exogenous FFA into TG was shown to be increased in diabetic dog hearts (76) *in vivo* and in myocardial homogenates (57). Similar results were found in perfused diabetic rat hearts (23, 53) and in studies using rat heart homogenates (63). While incorporation of exogenous FFA into TG was reported to be increased, oxidation was concomitantly decreased (23, 53). TG synthesis was enhanced even though glycolysis and glycerol phosphate content were decreased in the diabetic myocardium (18, 21, 66, 75).

The rate of myocardial TG lipolysis has been reported to be increased in alloxan diabetic rats (20, 30, 53, 83). In these studies, glycerol output was used as an index of lipolysis and diabetic hearts were perfused in the presence or absence of 0.5 mM exogenous FFA. However, the interpretation of these results is questionable since more recent studies have suggested that glycerol output may not be a quantitative index of lipolysis (13, 55) and exogenous FFA play a very important role in regulating TG lipolysis (12). Glycerol production has been claimed to be a valid index of lipolysis since the heart contains very little glycerol kinase and glycerol is not utilized by the heart (94). However, the possibility of other sources of glycerol, such as hydrolysis of α-glycerol phosphate, has not been adequately investigated in the heart (13, 30, 55). Studies by Crass et al. have shown that addition of exogenous FFA to perfused hearts markedly inhibited TG lipolysis. In diabetes serum FFA concentration is elevated (63, 78, 80). Thus, the increased rate of TG utilization shown in the above studies may be due to the withdrawal of inhibition of lipolysis by perfusing diabetic hearts with or without low exogenous FFA concentrations.

Uptake of exogenous FFA, plasma FFA or TGFA, will also affect the intracellular content of lipid intermediates, TG synthesis and lipolysis. Published data on the uptake of

exogenous long chain FFA by the diabetic heart has been variable. Uptake has been reported to be unchanged (23, 76), reduced (53) and increased (33, 95). Uptake of exogenous TGFA is dependent upon the enzyme lipoprotein lipase (LPL). Here, too, studies on LPL activity are not conclusive. In alloxan diabetes, LPL activity was reported to be increased (22, 49) while another study showed decreased LPL activity (1, 60). A decrease in LPL activity could be supported by the results of Kreisberg (53), who showed that the hydrolysis, uptake and oxidation of chylomicron ^{14}C-labeled TG by isolated perfused hearts of alloxan diabetic rats was decreased as compared to normal hearts.

Detrimental Effects of Abnormal lipid Metabolism on the Heart

Elevated levels of FFA in the blood may be detrimental to the ischemic heart function (42, 43, 68, 70, 80). In this context, a correlation between concentration of plasma FFA and mortality in patients with heart disease has been proposed (67). Experimental evidence showed that FFA have an arrhythmogenic effect on infarcted hearts (54). Elevated plasma FFA levels have also been shown to increase myocardial oxygen consumption (10) and decrease myocardial contractility in ischemia (38, 51, 52) and anoxia (43).

There have been several mechanisms suggested for the toxic effects of abnormal lipid metabolism on the heart.

An accumulation of tFFA may be one mechanism for the toxic effect of FFA on the heart. Kurien and Oliver (54) suggest that excess intracellular tFFA may react with ions to form soaps which have a nonspecific detergent effect on cell membranes and enzymes. In addition, a reduction in intracellular ion (K^+, Ca^{++}) concentration may alter membrane potentials and excitation-contraction processes.

Increased cellular concentrations of long-chain acyl CoA and long-chain acyl carnitine have been suggested to inhibit several cellular reactions (84). The reactions inhibited included are adenine nucleotide translocase, carnitine acyl transferase, NADH-NADP dehydrogenase, 3-phosphoglycerate dehydrogenase, tri-carboxylic carrier, acyl CoA synthetase, dicarboxylic carrier, α-ketoglutarate dehydrogenase, inorganic phosphase transport, and Na^+,K^+ ATPase.

TG accumulation, in itself, does not appear to be detrimental to the heart. However, an ultrastructure study by Giacomelli and Wiener (32) has suggested that excessive accumulation of lipid droplets in the diabetic myocardium leads to shrinkage and increased density of mitochondria. The mitochondria become surrounded by a single membrane. With time, the breakdown of mitochondria and lipid droplets continued until their conversion to large residual bodies by the process of autophagocytosis.

Myocardial Dysfunction in Diabetes

It is now well established that persons with diabetes mellitus have a greater incidence of cardiovascular diseases (48). It has been postulated that the myocardial disease was probably secondary to coronary vascular disease (97). However, a more recent study has shown that the rates of congestive heart failure in diabetic men and women are considerably higher than in nondiabetics. There was no correlation between the increased incidence of congestive heart failure and coronary heart disease (48).

A number of studies have shown changes in myocardial function in experimental and human diabetes exclusive of myocardial ischemia. Regan et al. (76) have shown evidence for altered mechanical performance in diabetic dogs which was attributed to increased stiffness of the left ventricle. The increased stiffness was associated with interstitial accumulation of glycoproteins, intracellular TG and cholesterol. Similar results were also found in human diabetes (77). An ultrastructure study by Giacomelli and Wiener (32) in hearts from genetically diabetic mice showed a number of pathological alterations. These changes included large numbers of lipid droplets, shrinkage and increased electron density of mitochondria, a single membrane around mitochondria, loss of filaments, vascular abnormalities and degenerative changes in perivascular nerve endings.

The observed alterations in cardiac muscle cells appeared to precede the development of vascular lesions. Thus, it appears from these data that diabetes has a direct pathogenic effect on the myocardium that is not related to preexisting coronary vascular disease.

Other studies on the diabetic myocardium have shown depressed Ca^{++} transport into the sarcoplasmic reticulum (73), marked inhibition of contractile protein ATPase activity (58) and reduced content of ATP (2, 71). Another study found impaired mitochondrial synthesis of high energy phosphate compounds in alloxan diabetic hearts (39). The inhibition of adenine nucleotide translocase and other enzymes by acyl CoA accumulation (discussed in the previous section) may be related to these abnormalities (84, 85).

Hearse demonstrated that the diabetic myocardium has an increased susceptibility to anoxic or ischemic damage. In diabetes, the ability of working rat hearts to withstand and recover from a period of severe ischemia (41) or anoxia (40) was depressed. Similar results were found in the global ischemic perfused heart (24). Haider et al. (36) found a substantial reduction of ventricular performance after regional ischemia in the diabetic dog as compared to control animals. Sinclair-Smith and Opie (86) found that the rate of release of lactate dehydrogenase (an index of the severity of ischemia) was greater from diabetic ischemic

hearts than nondiabetic ischemic hearts. Ketone bodies significantly increased the rate of enzyme release.

It is interesting to note that many of the abnormalities of lipid metabolism seen in the ischemic myocardium are similar to the lipid abnormalities of the diabetic heart shown in Table 1. Because the diabetic and ischemic myocardium have similar alterations in lipid metabolism, one might expect the magnitude of the changes of lipid metabolism to be greater in the diabetic ischemic heart than in nondiabetic ischemia. Feuvray et al. (24) found a larger increase in long chain acyl CoA and acyl carnitine content from diabetic ischemic hearts as compared to nondiabetic control ischemia. This response may be one explanation for the increased vulnerability of the diabetic myocardium to ischemic damage.

TABLE 1

ABNORMALITIES OF MYOCARDIAL LIPID METABOLISM IN EXPERIMENTAL ISCHEMIA AND DIABETES MELLITUS

Myocardial Lipid Abnormalities	Disease Process	
	Ischemia*	Diabetes*
Plasma FFA	↑35,67	↑63,78,80
FFA uptake	↑52,70	↑33,95
Intracellular FFA	↔82	↑28
Fatty acyl CoA	↑85,93	↑6,24
Carnitine	↓85	↓6,24
Acylcarnitine	↑85,93	↑6,24
Carnitine acyl transferase	↓85,93	↓6,24
Fatty acid oxidation	↓82	↓23,53
TG content	↑82,14	↑20,63,76,78
TG synthesis	↑82,96	↑63
TG lipolysis	↑9,92↓14	↑20,30,53,83↓
Lipoprotein lipase activity	↓50	↑22,49↓1,53,60

*References cited

↑Increase

↓Decrease

PART I

EFFECTS OF DIABETES ON COMPOSITION OF HEART LIPIDS

CHAPTER II

INTRODUCTION

There is an abundance of evidence which suggests that the metabolism of fatty acids, the preferred fuel for cardiac muscle, is altered in experimental and human diabetes. This is illustrated by the fact that an accumulation of myocardial TG occurs in experimental (20, 63, 76, 78) and human diabetes mellitus (77). Evidence has been presented (76) that alterations in myocardial lipid metabolism are accompanied by, but not necessarily related to, an alteration in mechanical performance of the myocardium. Other more recent studies on the diabetic myocardium have shown: 1) depressed mechanical performance in perfused hearts (62), 2) reduced content to ATP (2, 71), 3) inhibition of contractile protein ATPase activity (58), 4) depressed calcium transport in the sarcoplasmic reticulum (73), 5) altered ultrastructure of mitochondria and myofilaments (32), 6) increased vulnerability to anoxia (40) or ischemia (24, 41), and 7) an increased incidence of heart disease (48).

The mechanisms(s) underlying the accumulation of myocardial TG is not fully understood. The increase in

heart TG in the 48-hour alloxan-diabetic rat required growth hormone and cortisol (20) and occurred when the rat consumed a fat-free diet. Insulin treatment, in vivo, prevented the increase in heart TG (63, 78). Collectively, these studies suggested that increased deposition of myocardial TGFA was derived from enhanced mobilization of adipose tissue fatty acids. Several conflicting reports have shown that the uptake of exogenous FFA by diabetic hearts was either unchanged (23, 76), decreased (53), or increased (95). However, these studies have also shown that oxidation of exogenous FFA was decreased with commensurate enhancement of FFA incorporation into myocardial TG.

One factor which may contribute to the accumulation of TG in the diabetic myocardium is a change in the serum FFA composition and/or TGFA composition. Chain length and degree of unsaturation of exogenous FFA were shown by Evans (23) to affect uptake and metabolic fates, principally oxidation and incorporation into tissue lipids. As chain lingth of saturated fatty acids with an even number of carbon atoms increased, uptake and oxidation decreased while incorporation into tissue lipids increased. Uptake of oleic acid was greater than stearic or linoleic acid (23). Other studies have shown that oleic acid was incorporated preferentially into TG, whereas erucic and stearic acid accumulated in tFFA (91). Linoleic and linolenic acid

were found primarily in the phospholipid fractions. Palmitate was found chiefly as the carnitine derivative.

Since a change in heart TGFA, tFFA and/or sFFA composition may contribute to the accumulation of TG, the objective of the present study was to determine how streptozotocin-induced diabetes in rats affects the fatty acid composition of these lipids. To my knowledge, the composition of TGFA and tFFA in hearts from diabetic animals has not been described.

CHAPTER III

METHODS AND MATERIALS

Induction of Streptozotocin Diabetes

Male rats of the Sprague-Dawley strain (Holtzman Co., Madison, Wis.) weighing 250-300 g had free access to food and water. The rats were divided into three groups. The control group received neither streptozotocin nor insulin. Diabetic rats received streptozotocin (65 mg/kg) via the tail vein. It was dissolved in a 0.01 M citrate buffer, pH 4.5, and injected within 5 minutes. Insulin-treated diabetic rats received daily injections of 5 units isophane insulin suspension (Iletin, Eli Lilly Co.) subcutaneously beginning 48 hours after streptozotocin administration. The last insulin injection was given 24 hours prior to killing the rats. Duration of the diabetic state was 12 days. In one study four rats from each group were placed in metabolism cages. Body weight, water and food intake, and urine output were monitored daily. Urinary glucose and ketones were measured with Ames Keto-Diastix.

Tissue Analysis

After 12 days, each rat was anesthetized with ether. The chest was opened and the heart excised and placed in

cold bicarbonate buffer. The heart was mounted on a perfusion apparatus by cannulation of the aorta and perfusion-washed in retrograde fashion for 2 minutes with an oxygenated (95% O_2 - 5% CO_2) modified Krebs-Henseleit bicarbonate buffer containing 5.5 mM glucose. Hydrostatic perfusion pressure was 60 mm Hg. Immediately after perfusion, pieces of ventricle were freeze-clamped at the temperature of liquid nitrogen and weighed. Approximately 200 mg of the apex portion of the ventricle were placed immediately in boiling KOH. Glycogen was determined according to the method of Good, Kramer, and Somogyi (34). Final determination of glycogen as glucose equivalents was made using a Beckman Glucose Analyzer II.

Lipid analyses were performed on approximately 500 mg of frozen tissue from the midventricle. The tissue was freeze-clamped and the lipids extracted in 20 volumes of chloroform:methanol (2:1 v/v). After 24 hours, this extract was filtered through glass wool and dried at 37°C under nitrogen and partial vacuum. The lipids from the dried residue were dissolved in chloroform and quantitatively transferred to a silicic acid (Bio-Sil HA, minus 325 mesh, Bio-Rad Laboratories, Richmond, CA.) column. The neutral lipid plus free fatty acid (NL + FFA) fraction was eluted with 50 ml chloroform, and total phospholipid (PL) was separately eluted with 50 ml methanol. The NL + FFA

fraction was evaporated to dryness at 37°C under nitrogen and partial vacuum. The dried lipids were redissolved in 1.0 ml chloroform, and subjected to further fractionation by thin-layer chromatography (TLC). A portion (400 µl) of the NL + FFA fraction was applied to precoated silica gel G glass TLC plates, 20 x 20 cm (Applied Sciences Laboratories, State College, PA). A mixture of reference standards consisting of 1-monolein, 1,3-diolein, oleic acid, triolein, and cholesteryl oleate was applied to each plate. The TLC plates were chromatographed in a saturation chamber similar to that described by Parker and Peterson (72). The solvent system consisted of hexane:diethyl ether:acetic acid (120:47:1 v/v/v). This system provided resolution of the CE, TG and tFFA fractions. The TLC plates were air-dried at room temperature and the bands visualized with iodine vapor; each lipid fraction was then delineated. After dissipation of the iodine, the respective lipid fractions were scraped into teflon-capped tubes and the lipids were extracted from the silica gel with 10 ml chloroform-methanol (3:1 v/v). Tissue TG content was assayed using the method of Van Handel and Zilversmit (90). Total phospholipid content was determined from the 50 ml methanol column eluate using the method of Harris and Popat (37).

Dry:wet weight ratio was determined on a piece of frozen ventricle (basilar portion) dried to constant weight.

This ratio was then applied to all wet ventricle weights for conversion to respective dry weight values. All data were expressed per gram dry weight.

Serum Analysis

Immediately after excision of the heart, blood was pooled in the chest cavity and collected. The blood was allowed to clot and the serum obtained by centrifugation for 15 minutes at 2500 rpm. Serum glucose concentration was measured using a Beckman Glucose Analyzer II. Ames Ketostix were used to test for the presence of ketone bodies in the serum. sFFA were extracted in 5 ml of Dole extraction mixture (isopropanol:heptane:N H_2SO_4, 40:10:1 v/v/v). Two ml of heptane were added to the extract followed by 3 ml of distilled water. The heptane phase separated from the aqueous phase on standing. One ml of the heptane phase was taken for determination of total serum FFA concentration by the method of Itaya and Ui (47). A second 1.0 ml of the heptane phase was used for gas chromatographic analysis as described in the next section.

Gas Chromatographic Analysis of tFFA and sFFA

The fatty acid compositions of tissue TG, tFFA, sFFA were determined by gas chromatography. Triheptadecanoic acid was used as an internal standard for quantitative

analysis of TG, and heptadecanoic acid was the internal standard for tFFA and sFFA analyses. The TGFA composition was determined by taking 5.0 ml of the TG fraction after TLC plus the internal standard and evaporating the mixture to dryness under nitrogen at 37°C. The dried lipids were redissolved in 0.2 ml of benzene. The TGFA were transesterified to methyl esters by addition of 100 µl of 0.2N methanolic solution of (m-trifluoromethylphenyl) trimethylammonium hydroxide (Meth Prep II, Applied Science Laboratories). tFFA composition was determined by pooling FFA fractions from three hearts, adding internal standard, evaporating the mixture to dryness and redissolving the tFFA in 0.2 ml of benzene. An aqueous solution (200 µl) of (n-trifluoromethylphenyl) trimethylammonium hydroxide (Meth Prep I, Applied Sciences Laboratories) was added to the FFA to form trimethylammonium salts of the fatty acids. Two layers were formed with the aqueous layer containing the fatty acid salts on the bottom of the tube. A sample was withdrawn from the bottom layer and injected onto the gas chromatograph (Perkin-Elmer Sigma 3) where these salts were converted on column to fatty acid methyl esters. Serum FFA composition was measured from 1.0 ml of the heptane extract and analyzed by the same procedure as tissue FFA analysis. The methyl esters of fatty acids were analyzed on a 50 foot capillary column containing diethylene

glycol succinate. All runs were done isothermally with a column temperature of 180°C, and injector and detector temperatures of 225°C. Flow rate was 4 cm^3/min. The area corresponding to the individual fatty acids were measured using a Hewlett Packard Digitizer and were identified by comparing retention times to those of standards obtained from Alltech Associates, Inc. The data were expressed either as area percent of the total fatty acid or as μmoles per gram dry weight; the latter calculated by comparing the areas of the unknown and internal standard.

The Student's t-test was used to determine the statistical significance of differences between independent means. A result of $P<0.05$ was regarded as significant.

CHAPTER IV

RESULTS I

Diabetes mellitus was induced by intravenous administration of streptozotocin. Within 24 hours after streptozotocin administration, the rats showed a marked glucosuria and polyuria that was maintained throughout the twelve-day period. These effects were accompanied by polydipsia and polyphagia. Ketone bodies were found in the urine only in trace amounts on days three or four. The insulin-treated group also showed polyuria, glucosuria and polydipsia during the 48 hours after streptozotocin administration. With insulin treatment these effects were abolished.

Diabetic rats showed a significant ($P<0.001$) reduction in body weight as compared with control and insulin-treated diabetic rats which gained weight throughout the 12 day period (Table 2).

Diabetic rats showed a marked elevation of serum glucose and FFA (Table 2). Insulin-treated diabetic rats also showed a hyperglycemia and hyperlipidemia at the time of killing. This was due, perhaps, to the fact that these animals received their last insulin injection 24 hours prior to killing. The peak action of isophane insulin is

TABLE 2

EFFECTS OF STREPTOZOTOCIN-INDUCED DIABETES WITH OR WITHOUT INSULIN-TREATMENT ON BODY WEIGHT, SERUM GLUCOSE, SERUM FREE FATTY ACIDS (sFFA) AND HEART GLYCOGEN

Group	12-Day Body Wt. (g)	Glucose (mg/dl)	sFFA (mM)	Heart Glycogen (μmoles C_6/g dry wt.)
Normal	348 ± 5 (20)	173 ± 5 (20)	0.565 ±.021 (13)	159.9 ± 15.5 (8)
Diabetic	241 ± 6[a] (25)	518 ± 10 (28)	1.032 ±.071[a] (13)	216.4 ± 19.3[a] (10)
Diabetic + Insulin	339 ± 29[b] (11)	417 ± 29[a,b] (11)	1.282 ±.164[a] (3)	209.0 ± 11.9[a] (7)

NOTE: All values represent mean ± SE for the stated number of rats shown in parenthesis. Diabetes was induced by IV administration of 65 $mg \cdot kg^{-1}$ streptozotocin. The rats were killed after 12 days. Five units of isophane insulin suspension was administered daily (SC) beginning 48 hours after streptozotocin injection. The last injection was received 24 hours prior to killing.

[a] $P<0.05$ level of differences between experimental and normal mean values.

[b] $P<0.05$ level of differences between diabetic plus insulin and diabetic mean values.

approximately 2 to 8 hours. Therefore, at the time of killing, the insulin-treated diabetic rats were becoming insulin deficient.

Glycogen content increased in the diabetic myocardium. Insulin-treated diabetic rats also showed a similar increase in glycogen; again possibly due to a developing insulin deficiency during the 24 hours prior to killing.

Myocardial Lipid Content

Hearts from diabetic rats showed a three-fold increase in TG content relative to normal (Figure 2). Insulin treatment abolished this increase in TG even though the sFFA concentration was elevated in this group. In addition, tFFA were increased in diabetics as compared with normal rats. Insulin-treatment prevented the increase in tFFA, however only one pooled tFFA fraction was analyzed in this group. There was no difference in total phospholipid content in any of the three groups.

Composition of Myocardial Triacylglycerol Fatty Acids (TGFA)

Table 3 summarizes the percent composition of individual heart TGFA in normal, diabetic and insulin-treated diabetic rats. In normal hearts, the major TGFA were found to be palmitate (16:0), stearate (18:0), oleate (18:1) and linoleate (18:2), representing 36, 9, 23 and 19 percent of

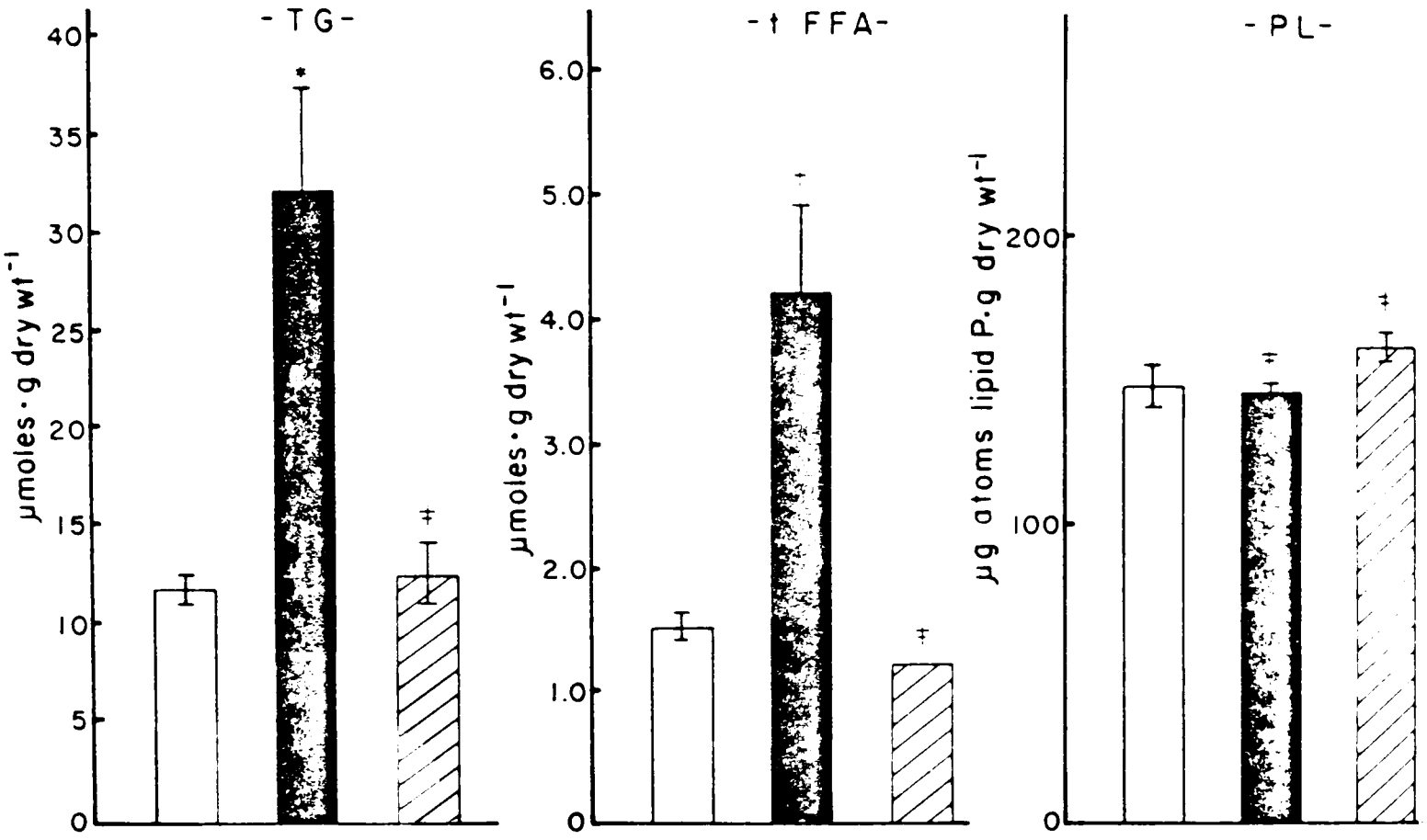

Figure 2: Effects of streptozotocin-induced diabetes on myocardial content of triacylglycerol (TG), free fatty acid (tFFA) and total phospholipid (PL). The clear bars represent control values, the solid bars diabetic values and the hatched bars insulin-treated diabetic values. Each bar represents the mean ± S.E. for 7-16 hearts except for tFFA of insulin-treated diabetic group in which only one pooled tFFA fraction was analyzed. *=$P<0.01$ with respect to control values. +=$P<0.05$ with respect to control values. ‡= not significantly different from control values.

TABLE 3

PERCENT COMPOSITION OF INDIVIDUAL HEART TRIACYLGLYCEROL FATTY ACIDS (TGFA) IN NORMAL, DIABETIC AND INSULIN-TREATED DIABETIC RATS

Fatty Acid	Percent of Total Triacylglycerol Fatty Acids		
	Normal (11)	Diabetic (13)	Diabetic + Insulin (7)
14:0	1.44 ± 0.36	1.44 ± 0.30	1.21 ± 0.21
16:0	35.60 ± 2.15	27.54 ± 1.13[a]	39.52 ± 3.06[b]
16:1	1.31 ± 0.20	0.71 ± 0.10[a]	2.01 ± 0.32[b]
18:0	8.98 ± 0.63	11.90 ± 0.60[a]	8.90 ± 0.94[b]
18:1	23.39 ± 1.99	25.55 ± 1.55	25.39 ± 1.23
18:2	18.90 ± 1.24	25.13 ± 0.63[a]	16.16 ± 1.62[b]
18:3	trace	trace	trace
20:0	trace	2.07 ± 1.24	trace
20:4	trace	trace	trace

NOTE: All values represent the mean ± SE for the stated number of rats shown in parenthesis.

[a] $P<0.05$ level of significance of differences between experimental and normal mean values.

[b] $P<0.05$ level of significance of differences between diabetic+insulin and diabetic mean values.

the total TGFA, respectively. Myristate (14:0), palmitoleate (16:1), and arachidate (20:0) were also detectable, each representing from 0.5 to 2.0 percent of the total fatty acid. Linolenate (18:3) and arachidonate (20:4) were not found consistently in TGFA in all hearts analyzed (trace).

In diabetic hearts, there was a significant decrease in the percent composition of palmitate and palmitoleate as compared to normal values (Table 3, $P<0.05$). On the other hand, stearate and linoleate showed a significant increase in percent composition ($P<0.01$). Insulin treatment of diabetic rats prevented this change in heart TGFA composition; the percent composition of all individual fatty acids in insulin-treated diabetic rats being equivalent to that of control hearts.

Figure 3 shows the content of palmitate, stearate, oleate and linoleate in normal and untreated diabetic myocardial TGFA. In diabetes, the content of all heart TGFA were significantly increased over normal values ($P<0.05$). However, not all of the fatty acids were increased to the same extent. For example, in hearts from diabetic rats, palmitate was increased 2.3-fold, while the observed increases in stearate, oleate, and linoleate were 3.9-, 3.0-, and 3.2-fold, respectively. This data suggests that diabetes promotes a shift in the fatty acid composition in TG from palmitate to a predominance of 18 carbon fatty acids.

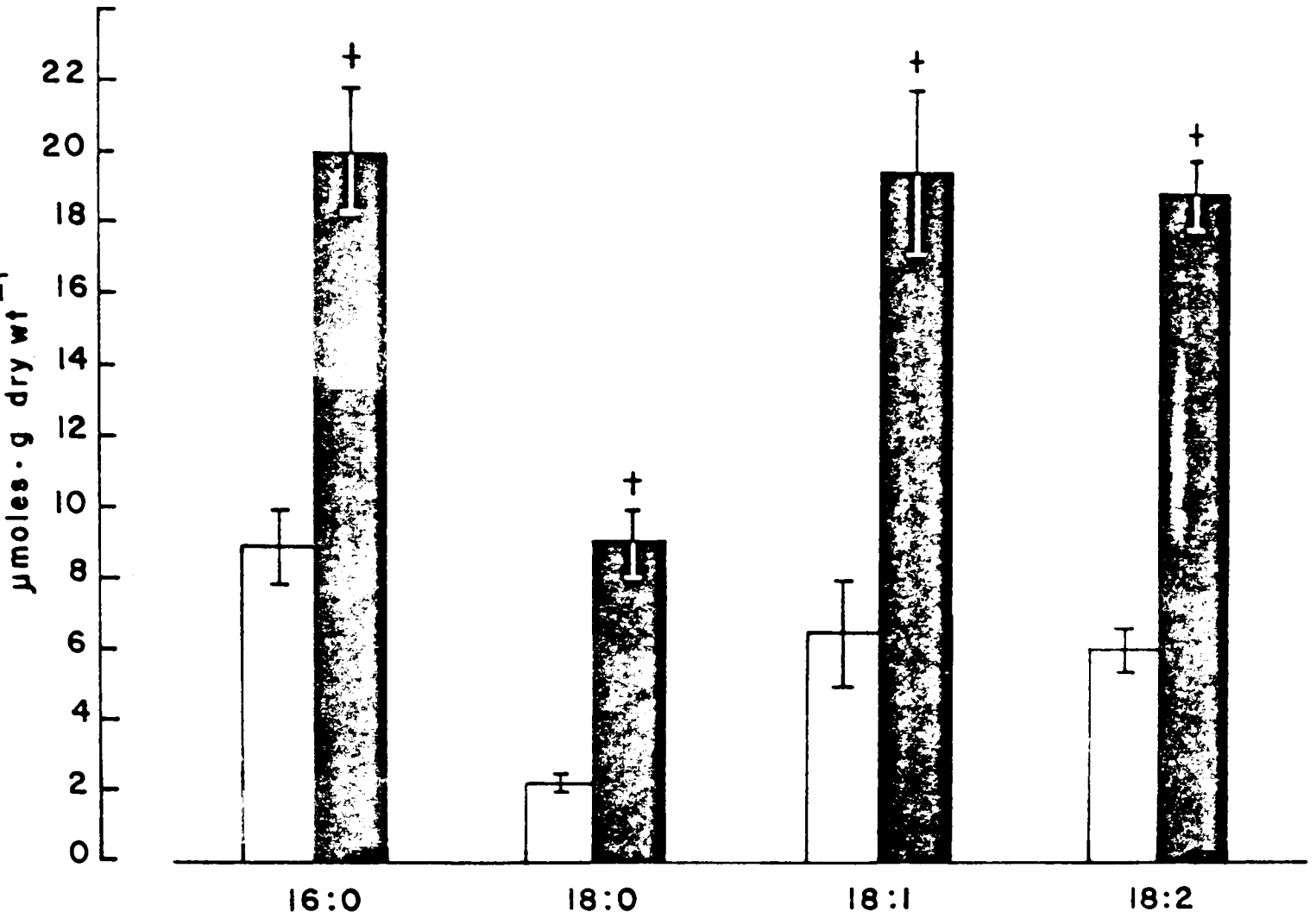

Figure 3: Effects of diabetes on palmitate (16:0), stearate (18:0), oleate (18:1) and linoleate (18:2) content of tissue triacylglycerol fatty acids (TGFA). The clear bars represent control values and the solid bars diabetic values. Each bar represents the mean ± S.E. for 10-11 hearts. † = $P<0.05$ with respect to control values.

Composition of Tissue Free Fatty Acids (tFFA)

In normal hearts, the major tFFA were palmitate, stearate, and oleate, each of which represented more than 20 percent of the total measureable tFFA (Table 4). Individually, the other tFFA did not exceed 2 percent of the total tFFA. The percent composition of tFFA in normal and diabetic rat hearts was similar with the exception of myristate, which was significantly reduced in diabetic hearts ($P<0.05$). The apparent reduction in palmitate and stearate percent composition and apparent increase in oleate and linoleate implied some directional changes in these fatty acids. The lack of statistical significance of these changes may have been due to the small number of observations and to the necessity for pooling of these tFFA fractions.

Composition of Serum Free Fatty Acids (sFFA)

In control rats, the sFFA composition was similar to the TGFA composition (Tables 3 and 5). In control animals, the major sFFA were palmitate, stearate, oleate and linoleate, representing 29, 9, 29, and 25 percent of the total sFFA, respectively (Table 5). Laurate, myristate, palmitoleate, linolenate, arachidate, and arachidonate were also present in sFFA, each constituting 0.3 to 2.4 percent of the total sFFA.

TABLE 4

PERCENT COMPOSITION OF TISSUE FREE FATTY ACIDS (tFFA) IN NORMAL AND DIABETIC RATS

Fatty Acid	Percent of Total Tissue Free Fatty Acids	
	Normal (3)	Diabetic (3)
14:0	1.95 ± 0.13	0.76 ± 0.18[a]
16:0	44.55 ± 3.55	35.99 ± 1.76
16:1	1.14 ± 0.11	1.04 ± 0.26
18:0	28.58 ± 3.05	24.33 ± 2.17
18:1	20.20 ± 5.26	33.63 ± 4.31
18:2	1.70 ± 0.86	2.11 ± 0.50
18:3	1.03 ± 0.19	0.66 ± 0.22
20:0	trace	trace
20:4	1.26 ± 0.60	1.34 ± 0.14

NOTE: All values represent mean ± SE for the stated number of rats shown in parenthesis.

[a]$P<0.05$ level of significance of differences between control and diabetic mean values.

TABLE 5

PERCENT COMPOSITION OF INDIVIDUAL SERUM FREE FATTY ACIDS (sFFA) IN NORMAL, DIABETIC, AND INSULIN-TREATED DIABETIC RATS

Fatty Acid	Percent of Total Serum Free Fatty Acids		
	Normal (10)	Diabetic (11)	Diabetic + Insulin (3)
12:0	1.47 ± 0.39	0.57 ± 0.12	trace
14:0	1.97 ± 0.31	1.81 ± 0.29	0.80 ± 0.09[a,b]
16:0	29.09 ± 0.94	27.65 ± 0.91	39.15 ± 1.31[a,b]
16:1	2.40 ± 0.26	0.91 ± 0.10[a]	2.46 ± 0.11
18:0	9.00 ± 1.33	11.02 ± 1.01	9.10 ± 0.50
18:1	29.26 ± 0.87	26.49 ± 0.80	27.12 ± 0.79
18:2	24.94 ± 0.70	28.60 ± 0.68[a]	21.08 ± 2.21[b]
18:3	0.52 ± 0.10	0.67 ± 0.09	trace
20:0	0.32 ± 0.06	0.40 ± 0.07	trace
20:4	0.82 ± 0.15	0.98 ± 0.22	trace

NOTE: All values represent the mean ± SE for the stated number of rats shown in parenthesis.

[a] $P < 0.05$ level of significance of difference between experimental and normal mean values

[b] $P < 0.05$ level of significance of difference between diabetic + insulin and diabetic mean values

The serum FFA composition of diabetic rats is shown also in Table 5. Palmitate composition in diabetic serum was comparable to that of normal serum. However, the diabetic rats showed a significant reduction in palmitoleate ($P<0.001$) and oleate ($P<0.05$). In addition, diabetic serum showed an apparent increase in stearate and a significant ($P<0.01$) increase in linoleate over normal values.

With respect to normal values, total sFFA concentration was elevated in insulin-treated diabetic rats (Table 2). Table 4 shows that the individual sFFA primarily responsible for this increase was palmitate. The percent composition of palmitate was significantly increased ($P<0.001$), while composition of the other sFFA in insulin-treated diabetic rats was similar to normal rats.

The mean concentrations ($\mu moles \cdot L^{-1}$) of palmitate, stearate, oleate, and linoleate in normal and untreated diabetic sera are shown in Figure 4. All of these sFFA were increased in diabetes with respect to control rats. Palmitate and oleate were increased 1.9-fold, whereas stearate and linoleate were increased 2.2- and 2.5-fold, respectively. Data from Table 4 and Figure 3 suggest that in diabetes there is a shift in the sFFA composition from 16 to 18 carbon fatty acids, primarily stearate and linoleate.

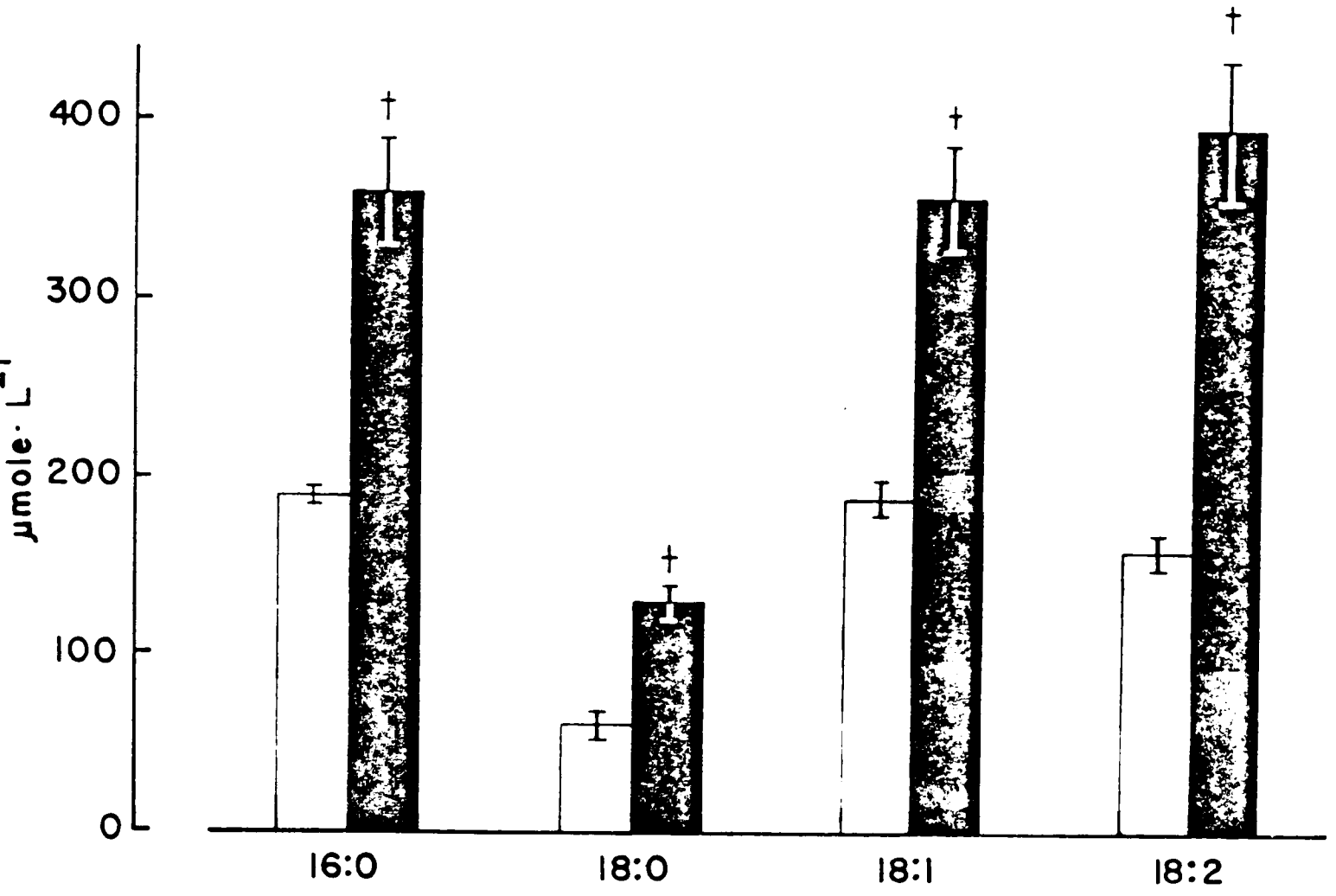

Figure 4: Effects of diabetes on serum concentration of palmitate (16:0), stearate (18:0), oleate (18:1) and linoleate (18:2). The clear bars represent control values and the solid bars diabetic values. Each bar represents the mean ± S.E. for 10-11 animals. + = $P<0.05$ with respect to control values.

CHAPTER V

DISCUSSION

The chronic 12-day streptozotocin-induced diabetic rat exhibited many of the signs characteristic of diabetes mellitus, i.e., polyuria, glucosuria, polydipsia and polyphagia. The rats were not ketoacidotic after days 3 and 4. The diabetic rats were markedly hyperglycemic and sFFA were elevated on day 12 at the time of killing. Diabetic rats lost weight during the 12-day period. Normal and insulin-treated diabetic rats showed a normal growth pattern. As reported in other models of experimental diabetes (18, 78), glycogen content was found to be increased in the 12-day streptozotocin model also. Insulin treatment of the diabetic rats prevented the polyuria, glucosuria, polydipsia, polyphagia and weight loss. But as indicated above, these rats were acutely hyperglycemic with elevated sFFA, thus reflecting some degree of insulin deficiency during the 24 hours after the last insulin injection.

A marked increase in triacylglycerol content is another characteristic of experimental diabetes (20, 63, 76, 78). The 12-day diabetic model presented in this

study also showed a marked increase in TG content. This was accompanied by an increase in tFFA levels. Insulin prevented the accumulation of TG and tFFA, despite the fact that sFFA concentration was elevated in this group. The increase in sFFA most likely developed during the 24 hours following the last insulin injection. Similar results were noted by Murthy and Shipp (63). These workers demonstrated that streptozotocin (60 $mg \cdot kg^{-1}$) diabetic rats treated with large doses of insulin for 5 days showed no increase in heart TG content after insulin withdrawal despite observed increases in sFFA and ketones. These workers also stated that a marked and sustained accumulation of myocardial TG was observed only in ketotic diabetic rats. Results from the present study did not support this contention. Although serum and urinary ketones were not examined in detail in the present study, Ames ketostix and ketodiastix do provide a reliable measure of the presence or absence of excess ketones (mainly acetoacetate). At the time of killing, ketone bodies were not detected in the urine or serum of the 12-day diabetic rats, although myocardial TG were significantly elevated.

The mechanism(s) responsible for the accumulation of TG in the diabetic myocardium are not known. Accumulation of TG in the heart could result from increased synthesis, decreased mobilization or both. Several studies have

suggested that TG synthesis in the diabetic myocardium was greatly enhanced. The incorporation of labeled exogenous FFA into TG was shown to be increased in diabetic dog hearts (76) *in vivo* and in myocardial homogenates. Similar results were found in perfused rat hearts and in studies on rat heart homogenates (57). While incorporation of exogenous FFA into TG was reported to be increased, oxidation was concomitantly decreased.

There are several factors which may contribute to an increase in TG synthesis in the diabetic myocardium. A relative deficiency of carnitine for transport of acyl CoA into the mitochondria is one factor. Bohmer *et al.* (6) and Feuvray *et al.* (24) reported that the content of myocardial free carnitine was reduced in alloxan diabetic rats. A deficiency of carnitine content could explain the decreased oxidation of exogenous FFA and, indirectly, the increased incorporation into heart TG. Another factor contributing to enhanced TG synthesis could be an increased synthesis of α-glycerophosphate, a substance normally derived from glycolysis, which can serve as a precursor of TG. The enzyme, α-glycerophosphate acyltransferase, has a lower Km for acyl CoA than that for carnitine acyl transferase (8). Formation of α-glycerophosphate could be increased in diabetes because of an excess of reducing equivalents in the myocardial cell. The malate-asparate

shuttle system which transports reducing equivalents from the cytosol into the mitochondria is reduced in alloxan diabetic rat hearts (74). Thus, increased formation of α-glycerophosphate would utilize excess reducing equivalents. However, the relative importance of this system is questionable since glycolysis is inhibited at the level of phosphofructokinase in diabetes (18). On the other hand, Denton and Randle (21) showed in hearts from alloxan diabetic rats, that despite decreased glucose uptake and α-glycerol phosphate concentration, flow of glucose carbon atoms through α-glycerol phosphate to TG was increased about threefold. A third factor that may aid increased synthesis of TG is a change in the composition of heart TGFA and sFFA. It has been previously shown that chain length and degree of unsaturation of exogenous FFA affect rates of uptake, oxidation and incorporation into tissue lipids of the perfused heart (23). For example, increasing the chain length of isotopically-labeled saturated fatty acids with an even number of carbon atoms resulted in decreased uptake and oxidation coupled with enhanced incorporation of label into tissue lipids. Interestingly, among the 18-carbon fatty acids, uptake and oxidation of oleic acid was greater than stearic or linoleic acid (23). Huxtable and Wakil (46) showed generally an inverse relationship between the rate of mitochondrial oxidation of

uniformly-labeled fatty acids and the degree of unsaturation; i.e., decreased oxidation with increased unsaturation. One exception was a higher oxidative activity with linoleic acid as compared with palmitic acid. The carnitine acyl transferase system has also been shown to have chain-length specificity (8); whereas, no preference was observed for β-oxidation of linoleic acid, oleic acid or palmitic acid (3, 4). Other studies have shown that preferential uptake of fatty acid does not occur in perfused rat hearts (87) or in dog hearts *in vivo* (61).

Results from the present study showed that both sFFA and myocardial TGFA have similar compositional changes in the 12-day diabetic model. The primary changes involved were a decrease in percent composition of 16-carbon fatty acids and a commensurate increase in the 18-carbon fatty acids, primarily stearate and linoleate. The change in composition was manifested both in percent composition and content data. According to the work of Evans (23), the ability of the heart to oxidize stearate and linoleate was less when compared with palmitate. Also, the incorporation of stearate and linoleate into tissue lipids was greater than palmitate. Therefore, it is possible that the described changes in sFFA and TGFA composition may be a contributing mechanism to the accumulation of TG seen in the diabetic myocardium.

Whether these changes in TGFA composition affects the activity of TG lipase, i.e., substrate acyl group specificity, and therefore mobilization of TG by the heart, needs to be assessed in future experiments. Mobilization of TG was reported to be enhanced in alloxan diabetic rat hearts (20, 30, 53, 83). Increased synthesis coupled with enhanced mobilization of endogenous TG in diabetes might indicate a wasting of energy by recycling TG.

The fact that the TGFA compositional changes and those of sFFA are directionally similar suggests that the source of elevated TGFA in diabetes may be from serum, but does not rule out myocardial chain lengthening and/or desaturation processes; possibilities which were not assessed in this study. Other studies (20, 63, 78) have suggested that the source of myocardial TGFA were from sFFA derived from enhanced mobilization of adipose tissue TG. In one report (20), the increase in heart TG in 48-hour alloxan-diabetic rats required growth hormone and cortisol and occurred despite the consumption of a fat-free diet. Insulin treatment prevented the increase in heart TG in the present study and in other studies (63, 78). The sFFA composition was also shown to change in human diabetes (80) and alloxan diabetic dogs (33). In human diabetics, the level of all sFFA were elevated with the greatest increases occurring in saturated and mono-unsaturated fatty acids (80). In

alloxan-diabetic dogs, all major fatty acids were increased and the percent composition of oleic and linoleic acids was significantly greater than that of normal animals (33).

In summary, the findings of the present study show that streptozotocin-induced diabetes results in an accumulation of TG by the diabetic myocardium. The increased cardiac content of TG was associated with a change in the TGFA composition. Insulin treatment of diabetic animals prevented the accumulation of TG and a change in composition. Serum FFA showed a similar compositional change. The shift in fatty acid composition of TGFA and sFFA was toward fatty acids that are less easily oxidized by the heart and are incorporated to a greater extent into complex tissue lipid. Therefore, it was suggested that these changes in composition might be a contributing mechanism for accumulation of TG by the diabetic myocardium.

PART II

EFFECTS OF DIABETES ON THE METABOLISM OF HEART LIPIDS

CHAPTER VI

INTRODUCTION

Diabetes mellitus is characterized, in part, by an accumulation of myocardial TG (20, 63, 76, 77, 78), tFFA (28, 30), long chain acyl CoA (6, 24, 30) and acyl carnitine (6, 24, 30). An increase in these lipids has been postulated to be associated with abnormal cardiac function (54, 76, 83). The mechanism(s) responsible for the accumulation of these lipids and metabolic intermediates is poorly understood. Studies on the diabetic rat heart have shown that TG synthesis (23, 53, 63) and lipolysis (20, 30, 53, 83) were enhanced. These results have led to the conclusion that there was an increased turnover of cardiac TG (53, 83). Opie (71) has termed this enhanced turnover as an increase in the activity of a TG-FFA cycle. The increased activity of this cycle was believed to cause a wasting of energy by reactivation of tFFA produced from lipolysis to long chain acyl CoA, followed by reincorporation into TG. This reaction converts one ATP to AMP plus pyrophosphate for every molecule of tFFA recycled. Increased activity of this cycle was used to explain the increased intracellular levels of TG, tFFA, and long chain

acyl CoA (71). Glycerol efflux from hearts perfused in the presence or absence of 0.5 mM exogenous FFA was used as an index of lipolysis. Glycerol production by the heart has been claimed to be a valid index of lipolysis, since the heart contains very little glycerol kinase activity and glycerol is not utilized by the heart (94). However, the possibility of other sources of glycerol, such as hydrolysis of α-glycerol phosphate, has not been adequately investigated in the heart (13, 30, 55). Another problem with the above studies on TG lipolysis was the level of exogenous substrates. FFA and glucose concentrations employed during in vitro perfusions were lower than the values found in diabetic serum. A study by Crass et al. showed that the concentration of exogenous FFA plays an important role in the regulation of TG lipolysis, i.e., elevated exogenous FFA will inhibit endogenous TG lipolysis in the perfused heart (12). In addition, other studies have shown that increased perfusate glucose concentration, such as those found in diabetes, have a sparing effect on TG mobilization (19, 31, 64). Therefore, the increased rate of TG utilization shown in the above studies may be due to the removal of inhibition of lipolysis by perfusing diabetic hearts with low levels of exogenous substrates.

The objective of the present study was to determine how 12 days of streptozotocin-induced diabetes affects the

rates of myocardial TG synthesis and lipolysis. Myocardial TG lipolysis was investigated using a technique of *in vivo* prelabeling of heart tissue lipids with palmitate-1-^{14}C followed by *in vitro* perfusion of the labeled heart. The disappearance of ^{14}C-labeled TG and chemically-determined TG during perfusion was used as an index of lipolysis. Since the level of exogenous FFA and glucose may exert an important regulation on TG lipolysis and synthesis, control and diabetic hearts were perfused with buffer containing the concentrations of glucose and FFA that are characteristically found *in vivo*. Myocardial FFA uptake and TG synthesis were assayed in the same heart by following the uptake and incorporation of exogenous [9, 10-^{3}H]-palmitate into TGFA. Endogenous lipid oxidation was measured by quantitatively measuring the metabolic $^{14}CO_2$ produced during perfusion.

CHAPTER VII

MATERIALS AND METHODS

Induction of Diabetes Mellitus

Diabetes was produced in male rats of the Sprague-Dawley strain (225-275 g) by injection of streptozotocin, 65 mg/kg iv, via the tail vein. Streptozotocin was dissolved in a 0.01 M citrate buffer pH 4.5 and was injected within 5 minutes. The duration of the diabetic state was 12 days. Control rats were untreated. All rats had free access to food and water throughout the experiment. Induction of diabetes was confirmed by measuring body weight, serum glucose and FFA concentrations, as described in Chapter IV. Results were similar to values obtained in Table 2.

Preparation of Palmitate-1-^{14}C-Serum Complex for Prelabeling Heart Lipids

The labeled fatty acid serum complex contained either 10 or 24 μcuries of palmitate-1-^{14}C (New England Nuclear Corp. Boston, Mass.) and 1 μmole of unlabeled carrier palmitate (lot no. 1394, Applied Sciences Laboratories, State College, Pa.) per ml of serum. A stock of solution

of 20 μcuries palmitate-1-^{14}C per ml of ethanol was prepared. The appropriate amount of this stock solution was added to unlabeled carrier palmitate. The solution was mixed with an aqueous solution of potassium carbonate. The resulting potassium-soap was evaporated to dryness under nitrogen on a hot plate. The soap was dissolved in 1 ml of saline and was added to chilled rat serum while being stirred rapidly with a magnetic stirrer. Another ml of saline was used to rinse.

In Vivo Prelabeling of the Heart Lipids with Palmitate-1-^{14}C

After 12 days the rats were weighed, anesthetized with ether, functionally hepatectomized and heart lipids prelabeled *in vivo* with palmitate-1-^{14}C. The procedure for functional hepatectomy and prelabeling of heart lipids was similar to that described by Borgstrom and Olivecrona (7). After an abdominal incision, the superior mesenteric artery was ligated, followed 2 minutes later by ligation of the hepatic artery and portal vein. After another minute, 0.5 ml of a palmitate-1-^{14}C-serum complex was injected slowly via a 1 cc tuberculin syringe and a 25-gauge needle into the inferior vena cava. Control rats received 5 μcuries while diabetic rats received either 5 or 12 μcuries of palmitate-1-^{14}C. The injected rat remained under light ether anesthesia for 30 minutes to allow

maximum incorporation of palmitate-1-^{14}C into the heart lipids. Thirty minutes after injection of the palmitate-1-^{14}C-serum complex, the chest of the rat was opened and the heart excised and mounted for perfusion

Preparation of Palmitate-1-^{14}C-Albumin Complex For Perfusion

Unlabeled palmitate was dissolved in 1.5 ml of absolute ethanol and then mixed with a solution of [9, 10-^{3}H]-palmitate (New England Nuclear, lot no. 1215-030). A potassium soap was formed by mixing the fatty acids with an aqueous solution of potassium carbonate. The potassium-soap was evaporated to dryness under nitrogen on a hot plate. The soap was redissolved in saline and added to cold Krebs-Henseleit bicarbonate buffer containing 10% w/v albumin (fatty acid-free; lot no. 97C-7440, Sigma Chemical Co.) while being stirred rapidly on a magnetic stirrer. The tube containing the potassium soap was rinsed twice with 2 ml of saline. The palmitate-1-^{14}C-albumin complex was then dialyzed overnight against substrate-free bicarbonate buffer in the cold. After dialysis, the fatty acid-albumin complex was added to the perfusion medium.

Composition of Recirculated Perfusion Medium and Experimental Groupings

The dialyzed fatty acid-albumin complex was added to a modified Krebs-Henseleit bicarbonate buffer. The final

concentration of albumin was 0.25 mM. Glucose was then added to the perfusate. The final concentrations of albumin, glucose, palmitate and [9, 10-^{3}H]-palmitate in normal and diabetic buffer are shown in Table 6. The concentrations of glucose and FFA used in normal and diabetic buffer were approximately equal to the values found in serum *in vivo*, shown in Table 2. The specific activities of both buffers were the same. During perfusion, the buffers were continuously gassed with 95% O_2 - 5% CO_2 and maintained at a temperature of 37°C.

Control rats were divided into three groups: initials, 30 and 60 minutes perfusion. Control hearts were perfused only with normal medium. Diabetic rats were similarly divided into initials, 30 and 60 minute perfusion groups. Diabetic hearts were perfused with diabetic medium. In addition, a small number of diabetic hearts were perfused with normal medium.

Preparation and Perfusion of Hearts

After prelabeling the heart lipids with palmitate-1-^{14}C, the chest was opened, the heart excised and placed in cold bicarbonate buffer. The heart was mounted on a perfusion apparatus by cannulation of the aorta and perfused in retrograde fashion to remove residual blood from the coronary vasculature. Control hearts were perfusion-

TABLE 6

COMPOSITION OF NORMAL AND DIABETIC BUFFER USED IN RECIRCULATED WORKING RAT HEART PERFUSIONS

Buffer	albumin mM	glucose mM	palmitate mM	[9,10-^{3}H]-palmitate µCi/100m l	specific* activity dpm/µmole
Normal	0.25	9.4	0.5	5	160,832 ±4,670
Diabetic	0.25	27.8	1.2	12	169,383 ±7,802

*The specific activity was determined by dividing the measured buffer radioactivity ([9,10-^{3}H]-Palmitate) by the titratable free fatty acid concentration.

washed with buffer containing 9.4 mM glucose; diabetic hearts were perfusion-washed with buffer containing 27.8 mM glucose. Free fatty acids were not present in these buffers.

In hearts from the initial groups, the perfusions were terminated after 5 minutes and the initial content and distribution of palmitate-1-^{14}C in tissue lipids were determined.

Hearts from the 30 and 60 minute perfusion groups were prelabeled and perfusion washed for 5 minutes as described above. During the washout period, the left atrium was cannulated. After washout, the hearts were perfused antegrade in a closed recirculating perfusion system (64) at 10 cm H_2O left atrial filling pressure. The aortic pressure and heart rate were monitored during perfusion. After 30 or 60 minutes of perfusion, the hearts were again perfusion washed for 2 minutes to remove residual labeled buffer from the coronary circulation and interstitial space. Lipid content, disappearance of palmitate-1-^{14}C from tissue lipids and incorporation of [9, 10-^{3}H]-palmitate into tissue lipids were determined. Chemically-determined and ^{3}H-labeled FFA uptake were measured also.

Collection and Analysis of $^{14}CO_2$

The perfusion apparatus was a closed system which permitted quantitative collection of $^{14}CO_2$ produced from

the oxidation of prelabeled heart lipids (15). Effluent gas containing unlabeled and labeled CO_2 was carried via tygon tubing to a series of three interconnected glass counting vials, each containing 8 ml of CO_2 trapping agent (ethylene glycol monomethyl ether:monoethanolamine, 7:1 v/v). Vials were changed at 15 minute intervals throughout perfusion. After termination of perfusion, the buffer was recirculated for 15 minutes and gassed with 100% nitrogen while the effluent CO_2 was trapped. Liquid phase $^{14}CO_2$ was also determined by acidifying an aliquot of each post-perfusate.

Tissue Analysis

Heart ventricular tissue was analyzed for content and radioactivity of lipids. Immediately after perfusion, pieces of ventricle were excised, clamped at the temperature of liquid nitrogen and weighed. Approximately 200 mg (wet wt.) of the apex portion of the ventricle was placed immediately in 1 ml boiling 30% KOH. Total tissue radioactivity was determined by counting duplicate 0.4 ml aliquots after addition of 5 ml CO_2 trapping reagent and 10 ml scintillation cocktail.

For estimation of tissue lipid content and radioactivity, approximately 400 mg of frozen tissue from the midventricle were crushed, weighed, and extracted in 20

volumes of chloroform: methanol (2:1 v/v). After 48 hr, the extract was filtered through glass wool and dried at 37°C under nitrogen and partial vacuum. The lipids from the dried residue were dissolved in chloroform and quantitatively transferred to a silicic acid (Bio-Sil HA, minus 325 mesh, Bio Rad. Laboratories, Richmond, CA) column. The neutral lipids plus free fatty acids (NL + FFA) fraction was eluted with 50 ml chloroform, and total phospholipid (PL) was eluted with 50 ml methanol. The NL + FFA fraction was evaporated to dryness at 37°C under nitrogen and partial vacuum. The dried lipids were redissolved in 1.0 ml chloroform and subjected to further fractionation by thin layer chromatography (TLC). A portion (460 µl) of the NL + FFA fraction was applied to precoated silica gel G glass TLC-plates, 20x20 cm (Applied Science Laboratories) in three bands consisting of six spots of 25 µl each. A mixture of reference standard consisting of 1-monolein,1, 3-diolein, oleic acid, triolein, and cholesterol oleate (Applied Science Laboratories) was applied to each plate. The TLC plates were chromatographed in a saturation chamber with a solvent system consisting of hexanes: diethyl ether: acetic acid (120:47:1 v/v/v). This system provided excellent resolution of the CE, TG, tFFA, diacylglyceride-cholesterol (DG-C) and MG fractions. The TLC plates were air dried at room temperature and the lipid bands visualized

with iodine vapor; each lipid fraction was then delineated. After dissipation of the iodine, the respective lipid fractions were scraped into Teflon-capped tubes and the lipids were extracted from the silica gel with 10 ml chloroform-methanol (3:1 v/v). Radioactivity was determined in 2 ml of each sample in duplicate. The sample was evaporated under nitrogen at 37°C. Ten ml of scintillation cocktail was added prior to counting. Other aliquots of each sample were assayed for tissue TG and tFFA content using methods described by Van Handel and Zilversmit (90) and Itaya and Ui (47), respectively. Total phospholipid content was determined on aliquots of the 50 ml methanol column eluate using the method of Harris and Popat (37).

Dry:wet weight ratio was determined on a piece of frozen ventricle (basilar portion) dried to constant weight. This ratio was then applied to all wet ventricle weights for conversion to respective dry weight values.

Perfusate Analysis

Chemically-determined and [9, 10-^{3}H]-palmitate uptake by the perfused heart were determined on duplicate 2 ml portions of initial and final perfusates. The FFA were extracted in 5 ml of Dole extraction mixture (isopropanol: heptane: N H_2SO_4, 40:10:1 v/v/v). Two ml of heptane and 3 ml water were added to each extract. The heptane phase separated from the aqueous phase upon

standing. One ml of the heptane phase was taken for determination of total serum FFA concentration by the method of Itaya and Ui (47). Another ml was delivered to a scintillation vial. The heptane was evaporated under nitrogen at 37°C, 10 ml of scintillation cocktail was added and the sample was counted.

Radioactivity Determinations

Tissue lipids, $^{14}CO_2$, and perfusate samples were counted on a Beckman LS-250 liquid scintillation spectrometer. The scintillator-solvent fluid consisted of 7.2 grams 2, 5-diphenyloxazole and 0.18 gram 1, 4-bis- 2-(4-methyl-5-phenyloxazolyl -benzene (Packard Instrument Co., Inc., Downers Grove, Ill.) per liter of toluene.

$^{14}CO_2$ vials were counted by the single isotope counting technique. Corrections for quench were made by the channels ratio method using standards obtained from Nuclear Chicago Corporation. Disintegrations per minute (dpm) were calculated using the following equation:

$$dpm = \frac{cpm\ (B) - bkg\ (B) \times 100}{\%eff\ ^{14}C\ in\ B}$$

bkg (B) = background in channel B

cpm (B) = counts per minute in channel B

% eff ^{14}C in B = efficiency of counting in channel B as determined from the quench curve

Perfusate and tissue lipid samples were counted using a double isotope counting technique. Since the spectra of ^{3}H and ^{14}C isotopes overlap, it was necessary to separate the counts due to ^{3}H in the ^{3}H channel (A) from those due to ^{14}C (B). The window settings of the pulse height discriminators were 0-300 in channel A and 300-540 in channel B. Quench curves using external standard-channels ratio method were determined for counting efficiency of ^{14}C in channel B, ^{14}C in channel A and ^{3}H in channel A using ^{3}H standards obtained from Amersham/Searle Corporation and ^{14}C standards obtained from Nuclear Chicago Corporation. Calculations for dual labeled counting are shown below.

$$\text{dpm } ^{14}C = \frac{\text{cpm (B) - bkg (B) x 100}}{\text{\% eff } ^{14}C \text{ in B}}$$

$$\text{dpm } ^{3}H = \frac{\text{cpm (A) - bkg (A) - dpm } ^{14}C \text{ x (\% eff } ^{14}C \text{ in A)}}{\text{\% eff } ^{3}H \text{ in A}}$$

cpm (B) = counts per min in channel B

cpm (A) = counts per min in channel A

bkg (B) = background counts in channel B

bkg (A) = background counts in channel A

% eff ^{14}C in B = % counting efficiency of ^{14}C in channel B

% eff ^{14}C in A = % counting efficiency of ^{14}C in channel A

% eff ^{3}H in A = % counting efficiency of ^{3}H in channel A

All samples were counted twice for 10 minutes.

Expression of Results and Statistical Analysis

All data were expressed per 100 mg dry weight of tissue. Mean values ± standard error were computed and the Student's t-test was used to determine the statistical significance of the difference between independent means. A result of $P<0.05$ was regarded as significant. Because of the small number of hearts in the diabetic group perfused with normal medium and the diabetic initial group that received 5 μCi of palmitate-1-^{14}C, no statistical and analyses were performed on these groups. Descriptive analysis was performed.

CHAPTER VIII

RESULTS

Initial Prelabeling of Heart Lipids with Palmitate-1-^{14}C

The distribution of radioactivity (^{14}C) in control and diabetic initial hearts prelabeled *in vivo* with palmitate-1-^{14}C is shown in Table 7. Control rats received 5 μCi of palmitate-1-^{14}C while diabetic rats were injected with 5 or 12 μCi. With the exception of CE and MG, less radioactivity was incorporated into all fractions of heart lipids of diabetic rats that received 5 μCi of palmitate-1-^{14}C than those of control hearts. Because of the decreased tissue lipid radioactivity in the diabetic hearts, the apparent specific activity of the TG and PL fractions (Table 8) were lower than control values. The decreased prelabeling of heart lipids from diabetic rats receiving 5 μCi of palmitate-1-^{14}C was due, perhaps, to isotopic dilution. The serum concentration of FFA in diabetic rats was 1.0-1.2 mM whereas that of normal animals was 0.5-0.6 mM.

Injection of 12 μCi of palmitate-1-^{14}C into diabetic rats resulted in a substantial increase in the radioactivity recovered in TG, tFFA, DG-C and CE fractions (Table 7). The

TABLE 7

RESULTS OF INITIAL PRELABELING OF HEART LIPID WITH PALMITATE-1-^{14}C

Group (n)	µCi palmitate -1-^{14}C	Lipid Fractions						Total Tissue Radioactivity	
		PL	TG	tFFA	DG-C	MG	CE	Lipid	KOH
Control (11)	5	18,480 ±3,570	120,480 ±13,342	3,544 ±578	6,544 ±1,117	453 ±112	134 ±21	149,581 ±17,907	121,720 ±13,713
Diabetic (4)	5	9,657 ±3,271	68,234 ±4,418	1,358 ±300	3,055 ±548	322 ±177	179 ±12	82,801 ±3,300	76,150 ±6,047
Diabetic (8)	12	16,348 ±2,107	218,026[a] ±16,586	6,362[a] ±728	10,233[a] ±643	439 ±70	742[a] ±79	252,150[a] ±17,616	257,520[a] ±6,462

NOTE: All values are expressed as dpm·100 mg dry wt^{-1} and represent the mean ± SE for the stated number of hearts (shown in parenthesis). Heart lipids were prelabeled in vivo with palmitate -1-^{14}C. The distribution of radioactivity in the lipid fractions and the total lipid and KOH extract radioactivity are shown. Abbreviations: PL=Phospholipids, TG=triacylglycerols, tFFA=tissue free fatty acids, DG-C=diacylglycerol-cholesterol, MG=monoacylglycerol, CE=cholesterol esters.

[a]$P<.05$, level of significance of difference between control rats that received 5µCi and diabetic rats that received 12µCi of palmitate-1-^{14}C mean values.

TABLE 8

INITIAL RADIOACTIVITY, CONTENT AND APPARENT SPECIFIC ACTIVITY (SA) OF MYOCARDIAL PHOSPHOLIPIDS (PL), TRIACYLGLYCEROL (TG) AND TISSUE FREE FATTY ACIDS (tFFA)

		PL			TG			tFFA		
Group (n)	µCi Palmitate -1-^{14}C	^{14}C dpm	Content µg atoms P	SA dpm·µg atom P^{-1}	^{14}C dpm	Content µmoles	SA dpm· µmole^{-1}	^{14}C dpm	Content µmoles	SA dpm· µmole^{-1}
Control (11)	5	18,480 ±3,570	16.52 ±0.48	1,130 ±250	120,312 ±13,342	1.57 ±0.15	80,947 ±9,659	3,544 ±578	0.73 ±0.08	4,012 ±389
Diabetic (4)	5	9,656[a] ±3,271	18.20 ±1.69	498[a] ±123	68,234[a] ±4,418	3.47[a] ±0.34	20,663[a] ±3,419	1,358[a] ±300	-----	-----
Diabetic (8)	12	16,348 ±2,107	14.62 ±0.88	1,137 ±151	218,0269[a] ±16,586	2.30[a] ±0.22	106,444 ±14,871	6,362[a] ±728	0.99[a] ±0.07	6,545[a] ±918

NOTE: All values are expressed on a 100 mg dry wt basis and represent the mean ± SE for the stated number of hearts (shown in parenthesis).

[a]P<.05 level of significance of difference between control rats that received 5µCi and diabetic rats that received 12µCi of palmitate-1-^{14}C mean values.

apparent specific activity of the TG fraction in this diabetic group was not significantly ($P>0.05$) different from control values receiving 5 μCi (Table 8). The PL specific activity of this diabetic group was also not different from control.

Total tissue radioactivity was determined from the KOH extract and is also listed in Table 7. In all three groups, the KOH extract radioactivity was not different from total tissue lipid radioactivity (the sum of all lipid fractions), thus indicating that recovery of radioactivity during extraction and lipid fractionation was approximately 100%.

Content of myocardial TG, PL and tFFA is shown in Table 8. The content of myocardial TG from both diabetic groups was greater than control value. However, there appeared to be a discrepancy in TG content between the two diabetic groups. The diabetic rats that received 5 μCi palmitate-1-^{14}C had a TG content greater than the diabetic rats that received 12 μCi palmitate-1-^{14}C. Myocardial PL content was slightly reduced in diabetic rats that received 12 μCi palmitate-1-^{14}C, but the reduction compared to the control value was not significant ($P>0.05$). The content of tFFA in diabetes was significantly increased ($P<0.05$) over control values. Because the specific activity of myocardial TG in control rats (5 μCi) and diabetic rats that received

12 μCi of palmitate-1-^{14}C were similar (Table 8), these two groups were used in all subsequent experiments.

Performance of Control and Diabetic Hearts During Perfusion

The performance and stability of control and diabetic working hearts are shown in Figure 5. These data were obtained from hearts perfused for 60 minutes with the buffer indicated (composition of buffers shown in Table 6). Diabetic hearts perfused with diabetic medium had a significantly lower heart rate ($P<0.05$) than did control hearts perfused with normal buffer. No differences in systolic and diastolic aortic pressures were observed between the three groups.

Mobilization of Endogenous ^{14}C-labelled TG

Table 9 shows the distribution of radioactivity in lipid fractions from initial, 30 and 60 minute control and diabetic hearts. In order to better illustrate the changes that occurred in tissue lipid radioactivity during perfusion, each fraction was plotted showing the changes in radioactivity after 30 and 60 minutes of perfusion. Figure 6 shows the disappearance of ^{14}C-labelled TG after 30 and 60 minutes of perfusion. Control TG radioactivity decreased by an average of 59,000 dpm·100 mg dry wt^{-1}

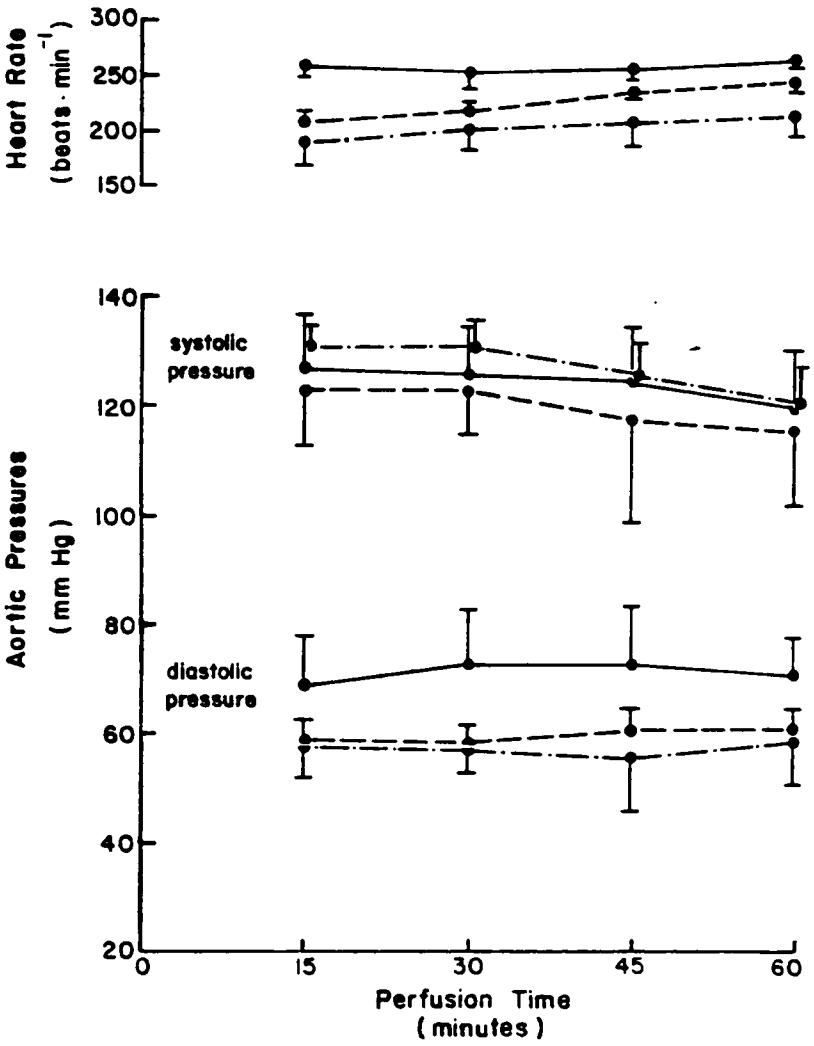

Figure 5: Heart rate, systolic and diastolic aortic pressures in control and diabetic hearts. Solid lines represent control hearts perfused with normal medium, dash lines diabetic hearts perfused with diabetic medium and dash-dot lines diabetic hearts perfused with normal medium. Each point represents mean ± S.E.

TABLE 9

DISTRIBUTION OF ^{14}C-RADIOACTIVITY IN LIPID FRACTIONS BEFORE AND AFTER RECIRCULATED PERFUSION OF CONTROL AND DIABETIC HEARTS

Group	Perfusion Time (n)	Lipid Fractions					
		PL	TG	tFFA	DG-C	MG	CE
Control							
	Initial (11)	18,480 ±3,570	120,312 ±13,342	3,544 ±578	6,149 ±1,117	453 ±112	134 ±21
	30 min (7)	18,784 ±2,810	60,823 ±13,552	2,884 ±655	4,490 ±1,289	313 ±198	267 ±54
	60 min (6)	13,965 ±2,643	32,031 ±4,836	1,545 ±104	2,350 ±295	92 ±38	171 ±53
Diabetic							
	Initial (8)	16,348 ±2,107	218,026[a] ±16,586	6,362[a] ±728	10,233[a] ±643	439 ±70	742[a] ±79
Diabetic buffer							
	30 min (7)	12,526 ±1,319	195,676[a] ±17,145	4,912[a] ±566	5,399 ±467	688 ±299	608[a] ±59
	60 min (7)	13,405 ±1,291	136,653[a] ±13,126	3,528[a] ±441	4,518[a] ±446	190 ±26	694[a] ±78
Normal buffer							
	30 min (4)	18,269 ±2,354	80,019 ±23,079	3,619 ±530	5,195 ±623	314 ±57	680 ±153
	60 min (2)	13,103 ±2,911	58,445 ±2,325	1,783 ±299	3,401 ±315	193 ±18	436 ±30

NOTE: All values are express as dpm·100 mg dry wt^{-1} and represent the mean ± SE for the stated number of hearts (shown in parenthesis). Hearts were prelabeled in vivo with palmitate -1-^{14}C and were perfused with or without recirculation for 30 and 60 minutes.

[a] $P<.05$, level of significance of difference between mean values from diabetic perfused with diabetic medium and control perfused with normal medium.

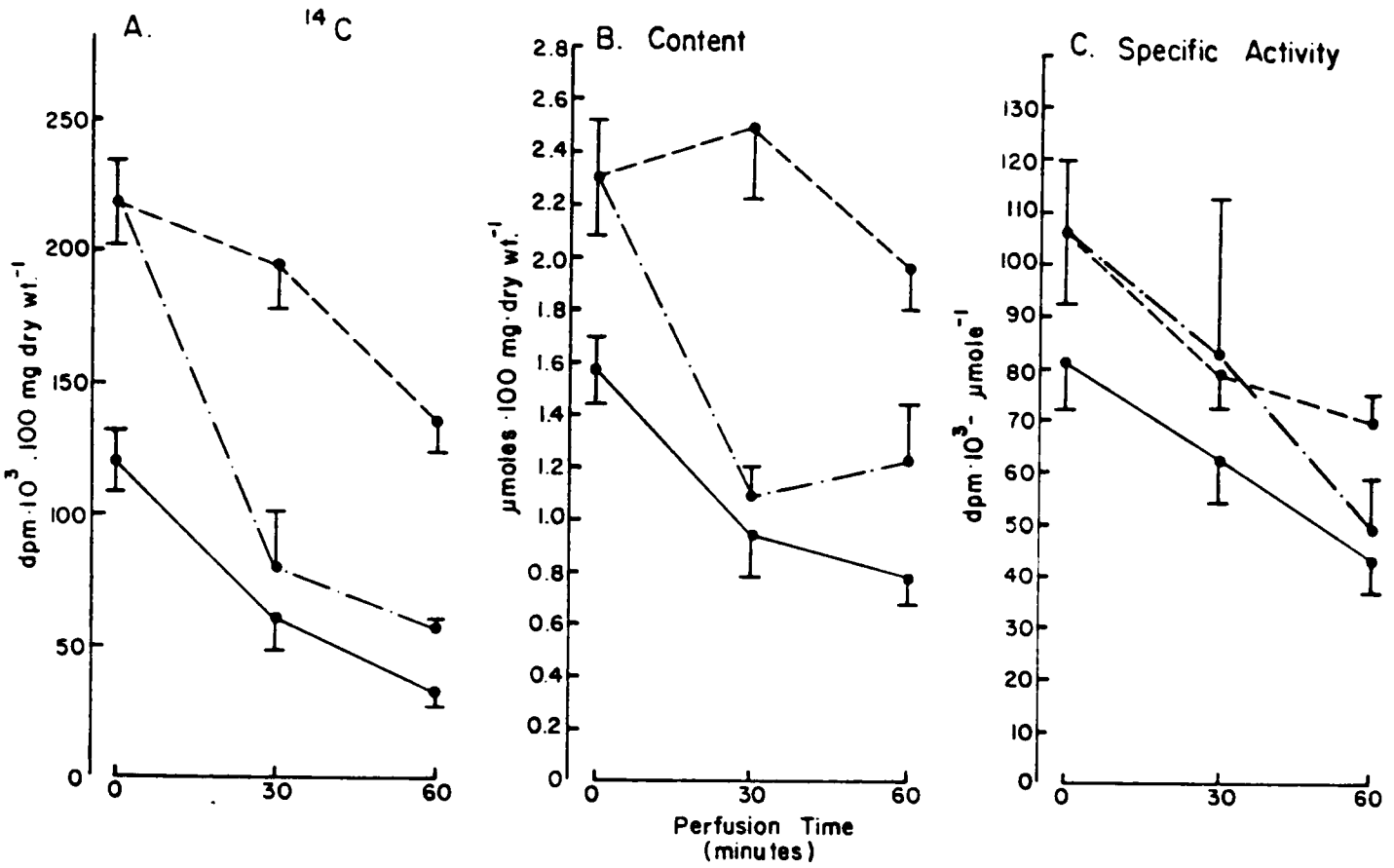

Figure 6: Changes in triacylglycerol ^{14}C-radioactivity, content and specific activity in control and diabetic hearts. Solid lines represent control hearts perfused with normal medium, dash lines diabetic hearts perfused with diabetic medium and dash-dot lines diabetic hearts perfused with normal medium. Each point represents the mean ± S.E.

during the first 30 minutes and a 29,000 dpm·100 mg dry wt^{-1} decrease during the second 30 minutes of perfusion. Dividing these values by the apparent specific activity of the TG (Table 10), an estimate of the μmoles·100 mg dry wt^{-1} of TG mobilized during these periods could be determined. Control hearts mobilized 0.735 μmoles·100 mg dry wt^{-1} of TG during the first 30 minutes of perfusion. During the next 30 minutes, the rate of mobilization was decreased to 0.460 μmoles·100 mg dry wt^{-1}. Diabetic hearts perfused with diabetic medium showed a mean decline in TG radioactivity of 22,350 dpm·100 mg dry wt^{-1} during the first 30 minutes. This decrease amounted to 0.210 μmoles·100 mg dry wt^{-1} of TG. During the second 30 minutes, 0.738 μmoles·100 mg dry wt^{-1} of TG were mobilized. In diabetic hearts perfused with normal buffer, TG lipolysis was greatly enhanced. The mean drop in radioactivity in this group during the first 30 minutes of perfusion was approximately 138,000 dpm·100 mg dry wt^{-1} or 1.2 moles·100 mg dry wt^{-1} of TG mobilized. During the next 30 minutes, the rate of TG lipolysis was decreased to 0.259 μmoles·100 mg dry wt.

Changes in PL, TG, and tFFA content and apparent specific activity before and after perfusion are shown in Table 10. In addition, Figure 6 shows the changes in TG content and apparent specific activity before and after perfusion. Control hearts had an average reduction in TG

TABLE 10

DISTRIBUTION OF RADIOACTIVITY, CONTENT AND APPARENT SPECIFIC ACTIVITY (SA) OF PHOSPHOLIPIDS (PL), TRIACYLGLYCEROL (TG) AND TISSUE FREE FATTY ACIDS (tFFA) BEFORE AND AFTER RECIRCULATED PERFUSION OF CONTROL AND DIABETIC HEARTS

Group	Perfusion Time (n)	PL			TG			tFFA		
		^{14}C dpm	Content μg atoms P	SA dpm·μg atom P^{-1}	^{14}C dpm	Content μmoles	SA dpm· $\mu mole^{-1}$	^{14}C dpm	Content μmoles	SA dpm· $\mu mole^{-1}$
Control										
	Initial (11)	18,480 ±3,570	16.52 ±0.48	1,130 ±250	120,312 ±13,342	1.57 ±0.15	80,947 ±9,659	3,544 ±578	0.73 ±0.08	4,012 ±389
	30 min (7)	18,781 ±2,810	16.40 ±0.57	1,147 ±190	60,823 ±13,552	0.94 ±0.16	62,596 ±8,178	2,884 ±655	0.87 ±0.14	2,519 ±811
	60 min (6)	13,965 ±2,643	17.49 ±0.35	886 ±172	32,031 ±4,836	0.78 ±0.10	43,196 ±6,562	1,545 ±104	0.75 ±0.04	2,041 ±64

TABLE 10--Continued

Group	Perfusion Time (n)	PL			TG			tFFA		
		^{14}C dpm	Content µg atoms P	SA dpm·µg atom P^{-1}	^{14}C dpm	Content µmoles	SA dpm· $\mu mole^{-1}$	^{14}C dpm	Content µmoles	SA dpm· $\mu mole^{-1}$
Diabetic										
	Initial (8)	16,348 ±2,107	14.62 ±0.88	1,137 ±151	218,026[a] ±16,586	2.30 ±0.22	106,444 ±14,871	6,362[a] ±728	0.99[a] ±0.07	6,545[a] ±918
Diabetic medium										
	30 min (7)	12,526 ±1,319	14.16[a] ±0.51	879 ±114	195,676[a] ±17,145	2.49[a] ±0.27	79,992 ±6,587	4,912[a] ±566	1.04 ±0.14	5,070[a] ±682
	60 min (7)	13,405 ±1,291	14.02[a] ±0.90	1,016 ±174	136,653[a] ±13,126	1.97[a] ±0.16	70,248[a] ±5,686	3,528[a] ±441	0.81 ±0.19	5,182[a] ±803
Normal medium										
	30 min (4)	18,269 ±2,354	12.95 ±0.68	1,447 ±254	80,019 ±23,079	1.09 ±0.12	83,213 ±29,946	3,619 ±530	0.94 ±0.07	3,953 ±551
	60 min (2)	13,103 ±2,911	15.41 ±0.19	852 ±199	58,445 ±2,325	1.23 ±0.22	49,430 ±10,619	1,783 ±299	0.86 ±0.25	2,143 ±277

NOTE: All values are expressed as mean ± SE on a 100 mg dry wt basis for the stated number of hearts (shown in parenthesis). Heart lipids were prelabeled in vivo with palmitate-1-^{14}C and perfused with or without recirculation for 30 or 60 minutes.

[a]$P<.05$, level of significance of difference between mean values from diabetic perfused with diabetic medium and control perfused with normal medium.

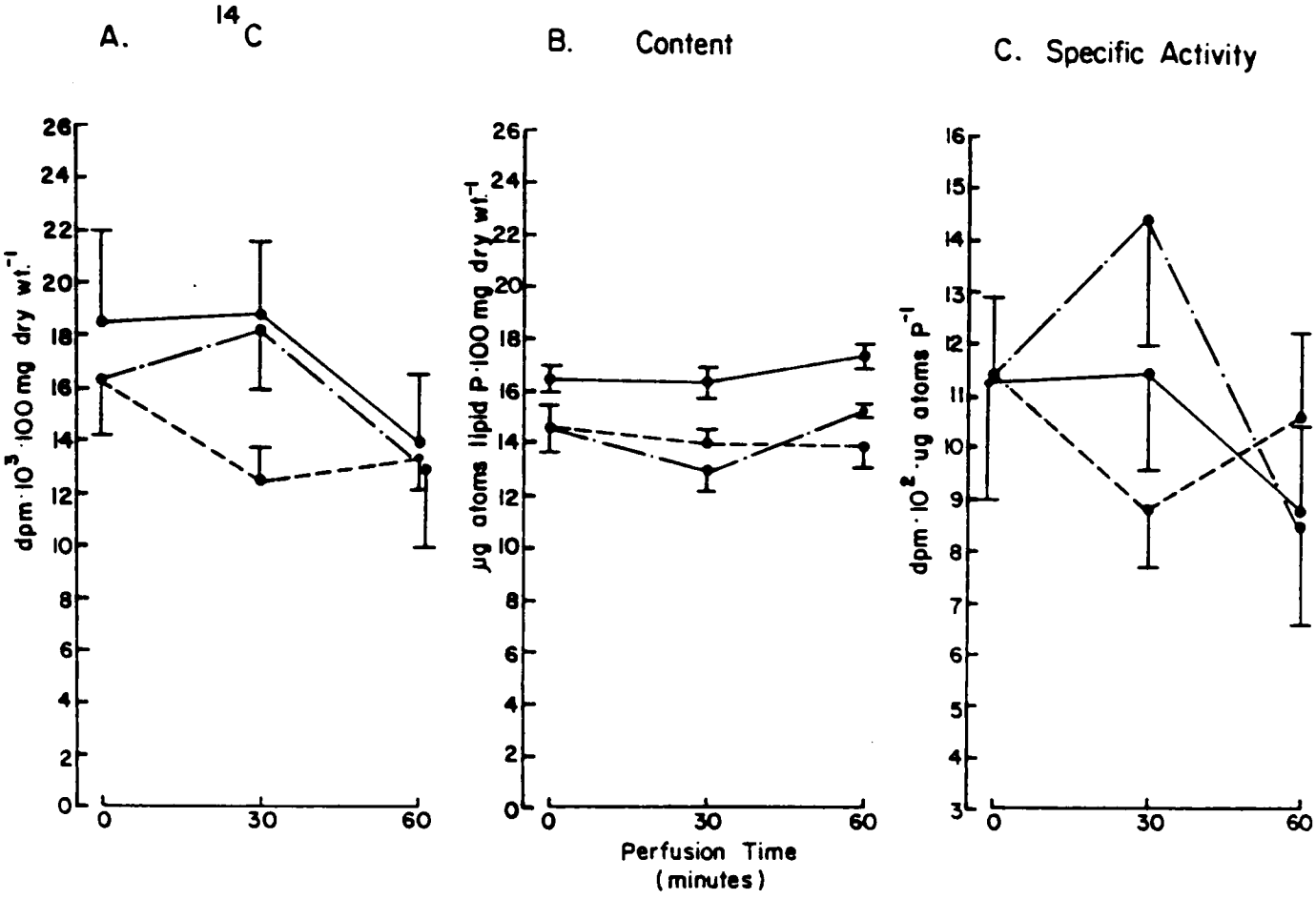

Figure 7: Changes in phospholipid ^{14}C-radioactivity, content and specific activity in control and diabetic hearts. Solid lines represent control hearts perfused with normal medium, dash lines diabetic hearts perfused with diabetic medium and dash-dot lines diabetic hearts perfused with normal medium. Each point represents the mean ± S.E.

content of 0.630 μmoles·100 mg dry wt^{-1} during the first 30 minutes of perfusion. This value was very close to the calculated decrease in TG content; determined by dividing the change in TG radioactivity by the apparent specific activity. During the next 30 minutes, there was only a small decrease in TG content (0.16 μmoles·100 mg dry wt^{-1}). TG apparent specific activity in control hearts decreased linearly throughout the perfusion. In diabetic hearts perfused with diabetic medium, TG specific activity decreased also but chemically-determined TG content was maintained throughout the perfusion period. TG content in diabetic hearts perfused with normal buffer declined by 1.21 μmole·100 mg dry wt^{-1} during the first 30 minutes of perfusion. This value is equivalent to the calculated decrease in TG content shown previously. No further decrement was seen during the next 30 minutes. The specific activity decreased in a linear fashion.

Mobilization of Endogenous ^{14}C-labelled PL

Tissue PL radioactivity (^{14}C), content and specific activity showed very little change during perfusion in the three groups (Figure 7). The initial PL content in diabetic hearts was lower than control values but not significantly ($P>0.05$). However, PL content in diabetic hearts was significantly ($P<0.05$) lower than control levels after

perfusion. Small decreases in radioactivity were evident in all groups after 60 minutes of perfusion.

Utilization of Endogenous ^{14}C-labeled tFFA

The tFFA content and radioactivity decreased in all three groups after perfusion (Figure 8). The largest decrease in radioactivity was in diabetic hearts perfused with normal medium. Specific activity decreased only in control and diabetic hearts perfused with normal medium.

Utilization of ^{14}C-Labeled DG-C, MG and CE

The disappearance of radioactivity (^{14}C) in DG-C, MG and CE of control and diabetic hearts are shown in Figure 9. During perfusion, radioactivity was lost from the DG-C and MG fractions, but not from the CE fraction.

^{14}C-labeled DG-C disappearance in diabetic hearts perfused with diabetic medium was similar to diabetic hearts perfused with normal medium. MG showed an increase in radioactivity during the first 30 minutes, but during the next 30 minutes there was a decrease.

Oxidative Metabolism of ^{14}C-Labeled Lipids; Release of ^{14}C-FFA Into the Perfusion Medium

Diabetic hearts perfused with diabetic medium and control hearts perfused with normal medium produced

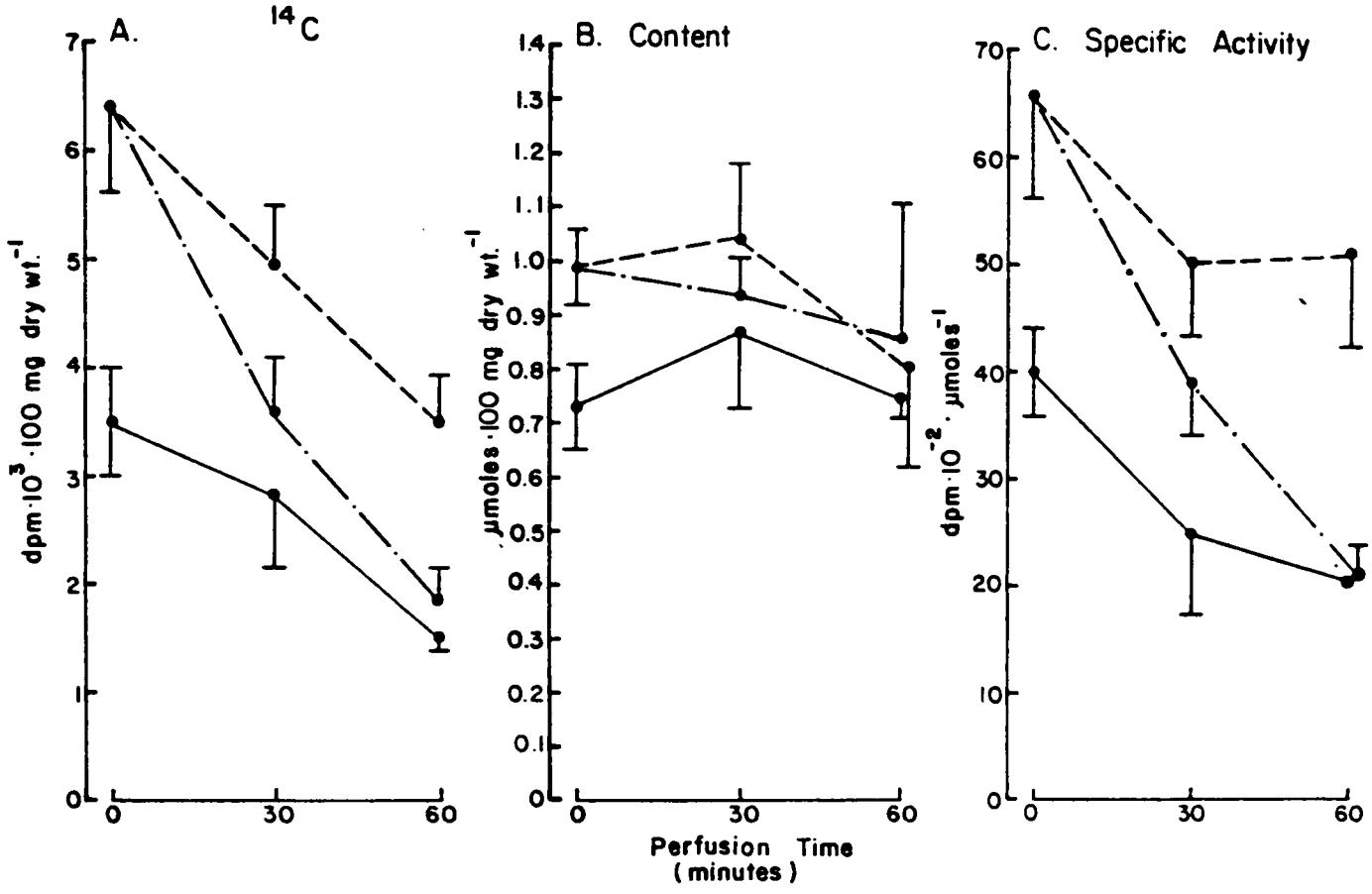

Figure 8: Changes in tissue free fatty acid ^{14}C-radioactivity, content and specific activity in control and diabetic hearts. Solid lines represent control hearts perfused with normal medium, dash lines diabetic hearts perfused with diabetic medium and dash-dot lines diabetic hearts perfused with normal medium. Each point represents the mean ± S.E.

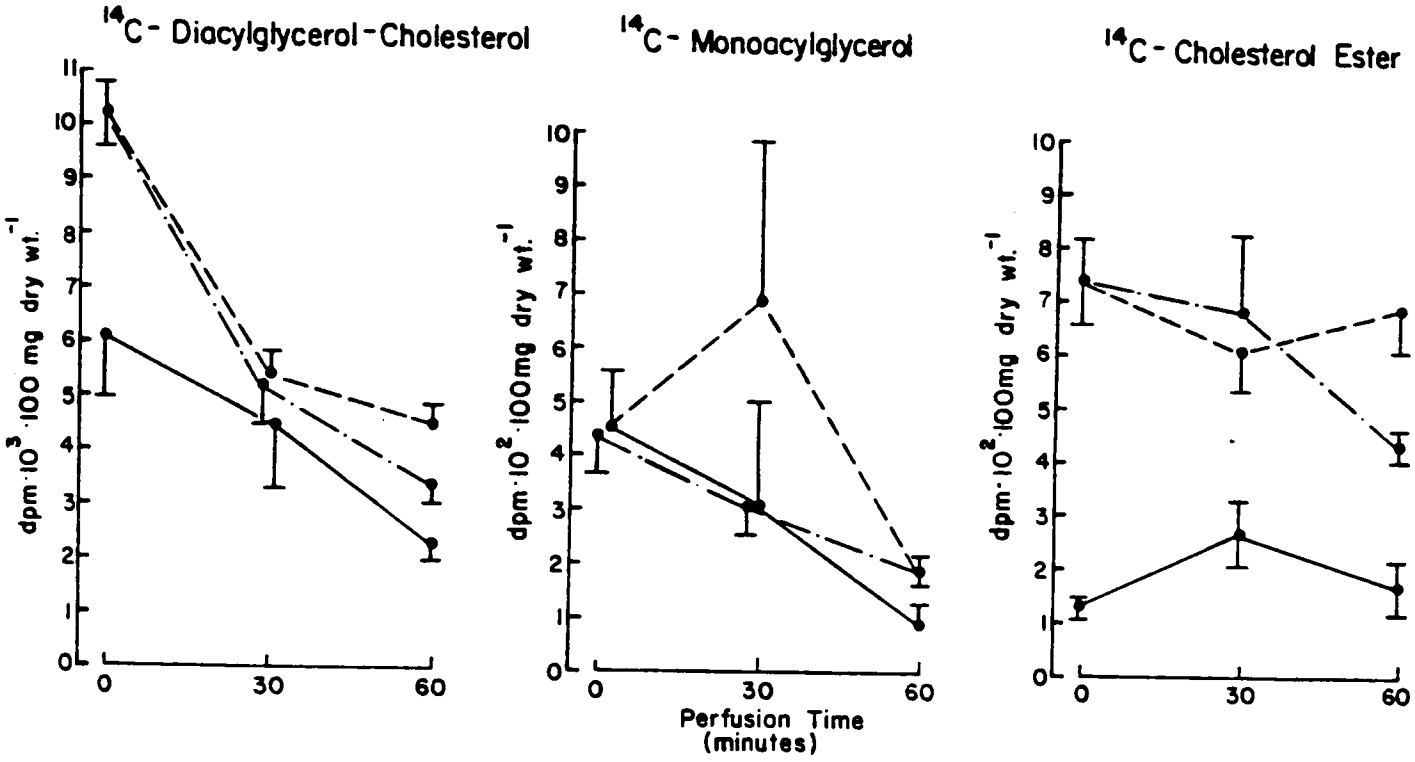

Figure 9: Changes in diacylglycerol-cholesterol, monoacylglycerol and cholesterol ester ^{14}C-radioactivity in control and diabetic hearts. Solid lines represent control hearts perfused with normal medium, dash lines diabetic hearts perfused with diabetic medium and dash-dot lines diabetic hearts perfused with normal medium. Each point represents the mean ± S.E.

quantitatively similar amounts of $^{14}CO_2$ (Figure 10). In contrast, diabetic hearts perfused with normal medium produced significantly greater quantities of $^{14}CO_2$. Similar results were obtained for total $^{14}CO_2$ (gas plus liquid phase $^{14}CO_2$) produced (Table 11).

Table 11 shows that the values for the production of $^{14}CO_2$ plus the release of ^{14}C-labeled FFA into the perfusion medium correlated with the disappearance of radioactivity of ^{14}C-labeled lipids. In all three groups, the differences in total lipid radioactivity between initial and perfusion groups were approximately equal to the sum of total $^{14}CO_2$ and ^{14}C-FFA released by the hearts into the perfusion medium. The drop in total ^{14}C-labeled lipid radioactivity was greatest in diabetic hearts perfused with normal medium. Both diabetic groups released more ^{14}C-FFA into the perfusion medium than controls. A test was run to determine if the ^{14}C-radioactivity could be due to $^{14}CO_2$ contamination during the extraction procedure. An aliquot of final perfusate was acidified and gassed with nitrogen for 1 hour. The radioactivity of the buffer was then determined and its presence in the perfusate confirmed after this procedure.

FFA Uptake In Perfused Hearts

Figure 11 shows FFA uptake in perfused control and diabetic hearts. Diabetic hearts perfused with diabetic

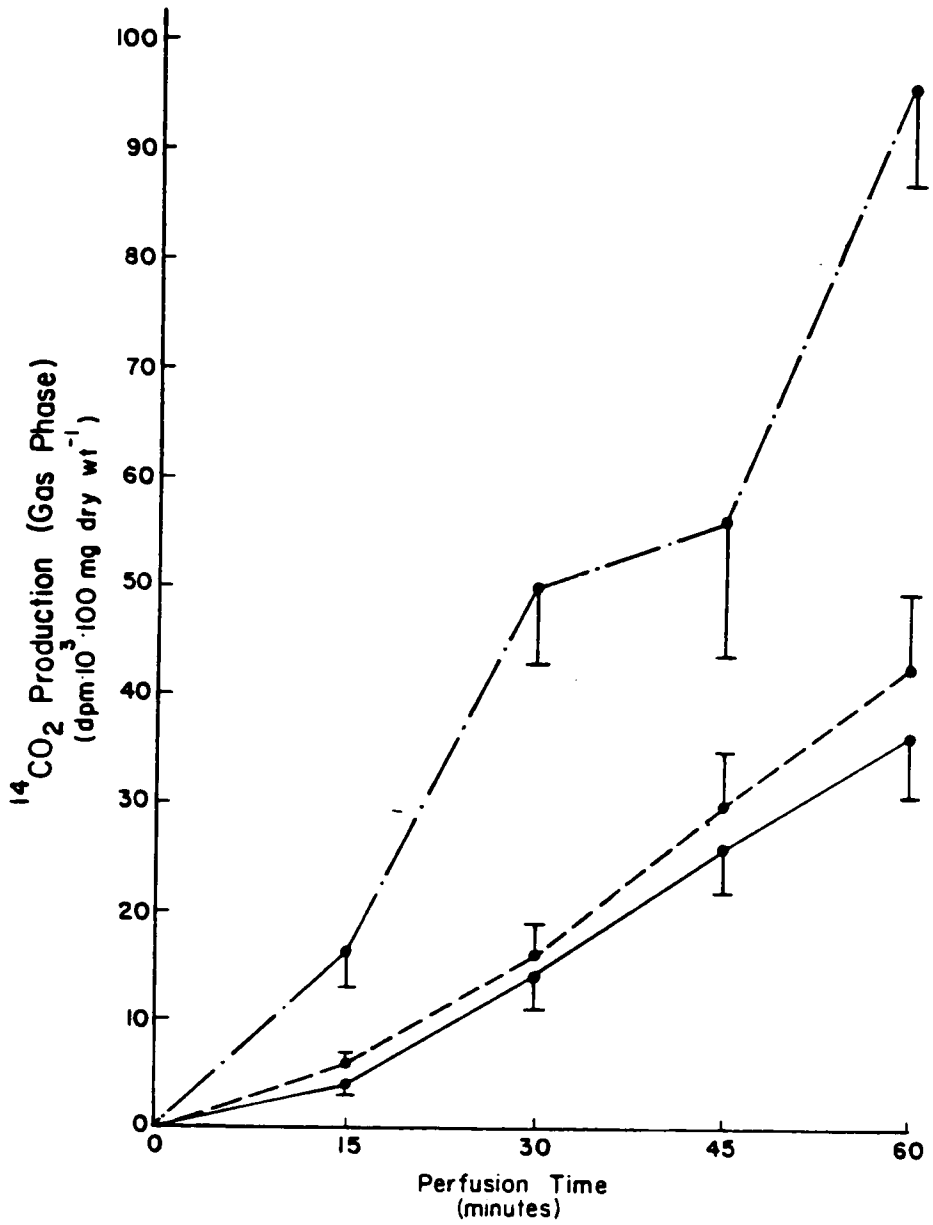

Figure 10: $^{14}CO_2$ production from control and diabetic hearts. Solid lines represent control hearts perfused with normal medium, dash lines diabetic hearts perfused with diabetic medium and dash-dot lines diabetic hearts perfused with normal medium. Each point represents the mean ± S.E.

TABLE 11

TOTAL TISSUE LIPID RADIOACTIVITY, TOTAL $^{14}CO_2$ PRODUCTION AND ^{14}C-FFA RELEASE FROM CONTROL AND DIABETIC HEARTS DURING PERFUSION FOR 30 AND 60 MINUTES

Group	Perfusion Time (n)	Total of Lipid Fractions	Total $^{14}CO_2$ Production	^{14}C-FFA Release
Control				
	Initial (11)	149,581 ±17,907		
	30 min (7)	86,921 ±17,241	31,089 ±3,768	3,649 ±990
	60 min (6)	50,159 ±6,251	53,504 ±7,387	5,643 ±1,379
Diabetic				
	Initial (8)	252,150[a] ±17,616		
	Diabetic medium			
	30 min (7)	219,809[a] ±18,136	27,033 ±4,499	30,635[a] ±6,170
	60 min (7)	158,342[a] ±12,785	63,390 ±10,105	63,661[a] ±9,032
	Normal medium			
	30 min (4)	108,098 ±24,338	120,775 ±15,028	33,348 ±5,177
	60 min (2)	77,363 ±5,863	158,783 ±4,217	48,135 ±6,664

NOTE: All values are expressed as dpm·100 mg dry wt^{-1} and represent the mean ± SE for the stated number of hearts (shown in parenthesis).

[a]$P<.05$ level of significance of difference between mean values from diabetic hearts perfused with diabetic medium and control hearts perfused with normal medium.

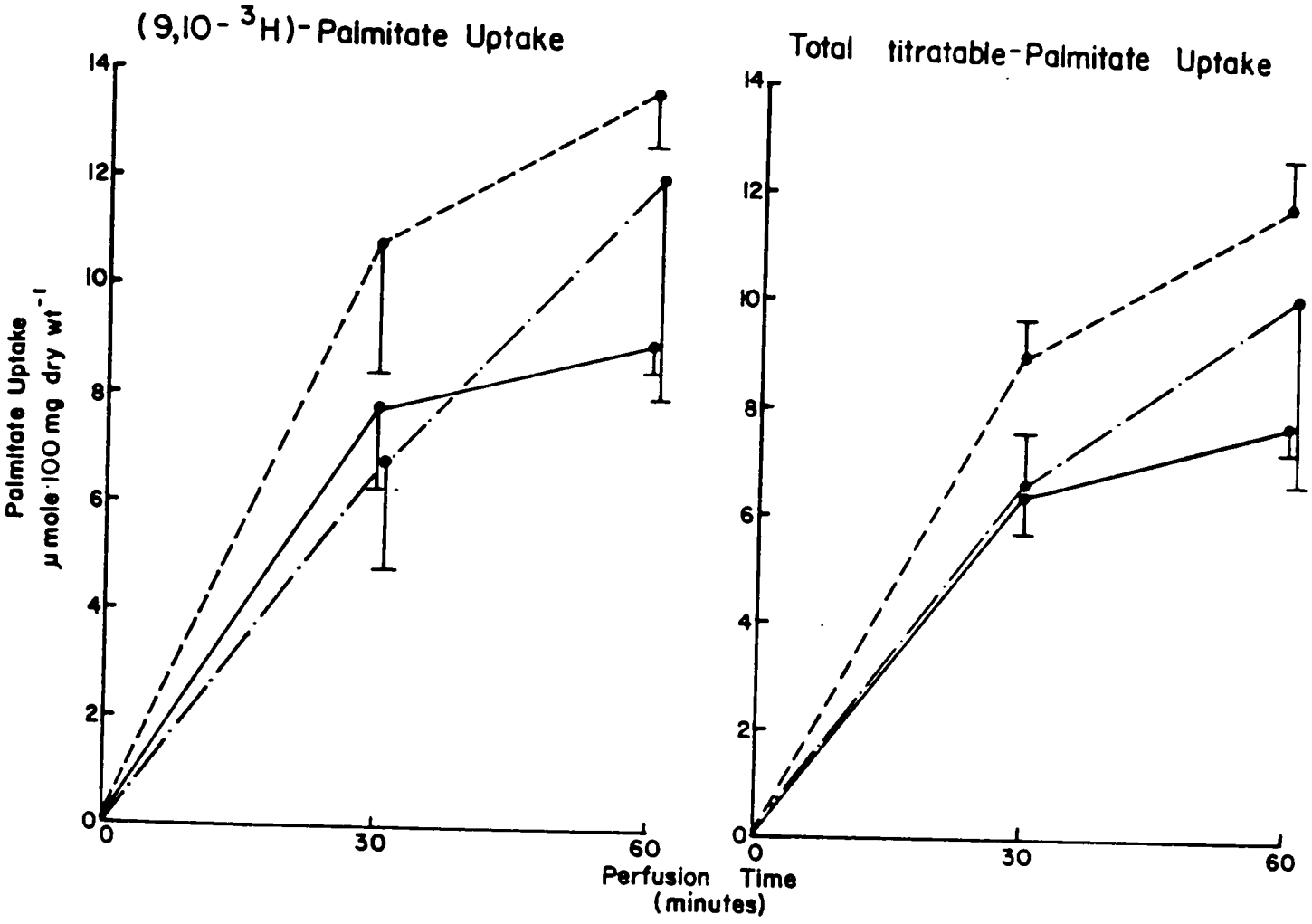

Figure 11: Total titratable and [9,10-^{3}H]-palmitate uptake in control and diabetic hearts. Solid lines represent control hearts perfused with normal medium, dash lines diabetic hearts perfused with diabetic medium and dash-dot lines diabetic hearts perfused with normal buffer. Each point represents the mean ± S.E.

medium had a significantly greater ($P<0.05$) FFA uptake than did control hearts. Diabetic hearts perfused with normal medium also had a lower FFA uptake. Both titratable and ^{3}H-FFA uptake gave similar results. Based upon perfusate specific activity, ^{3}H-FFA uptake on a micromolar basis was slightly higher than titratable FFA uptake.

Incorporation of Exogenous ^{3}H-Labeled Into Tissue Lipid

Incorporation of perfusate [9, 10-^{3}H]-palmitate radioactivity into tissue lipids of control and diabetic hearts are shown in Table 12. Incorporation into individual lipid fractions can be seen in Figures 12 and 13. In diabetic hearts perfused with diabetic medium, the incorporation of exogenous ^{3}H-labeled FFA into TG, tFFA, DG-C, MG and CE was greater than that seen in control hearts perfused with normal medium. Incorporation of exogenous ^{3}H-labeled FFA into PL was not enhanced in diabetic hearts perfused with diabetic medium as compared to control hearts. However, the incorporation of exogenous ^{3}H-labeled FFA into PL was greater in these two groups as compared to diabetic hearts perfused with normal medium. Incorporation of exogenous FFA into tissue lipids on a micromolar basis can be seen in Table 13. Incorporation was calculated by dividing ^{3}H-dpm of the lipid fraction (Table 7) by the perfusate

TABLE 12

INCORPORATION OF PERFUSATE RADIOACTIVITY ([9,10-^{3}H] Palmitate) INTO TISSUE LIPIDS OF CONTROL AND DIABETIC HEARTS AFTER 30 AND 60 MINUTES OF PERFUSION

Group	Perfusion Time (n)	Lipid Fractions						
		PL	TG	tFFA	DG-C	MG	CE	Total
Control								
	30 min (7)	13,556 ±2,276	30,812 ±6,267	5,125 ±259	5,130 ±799	380 ±125	3,064 ±237	57,280 ±8,790
	60 min (6)	17,642 ±1,794	62,285 ±19,210	6,101 ±677	5,119 ±781	462 ±205	2,091 ±396	93,702 ±21,170
Diabetic								
Diabetic Medium								
	30 min (7)	13,292 ±2,771	155,198[a] ±30,812	19,309[a] ±3,993	11,127[a] ±1,178	1,910 ±929	7,372[a] ±569	218,177[a] ±24,294
	60 min (7)	19,958 ±2,346	211,538[a] ±19,462	16,602[a] ±1,679	12,407[a] ±1,009	932 ±122	9,729[a] ±695	269,379[a] ±21,577
Normal Medium								
	30 min (4)	5,382 ±629	34,262 ±2,792	5,290 ±358	4,185 ±144	609 ±93	1,482 ±57	51,211 ±2,347
	60 min (2)	8,788 ±1,316	50,881 ±59	5,034 ±786	6,499 ±934	633 ±108	2,169 ±126	74,004 ±150

NOTE: All values are expressed as dpm·100 mg dry wt^{-1} and represent the mean ± SE for stated number of hearts (shown in parenthesis).

[a]$P<.05$, level of significance of difference between mean values from diabetic hearts perfused with diabetic medium and control hearts perfused with normal medium.

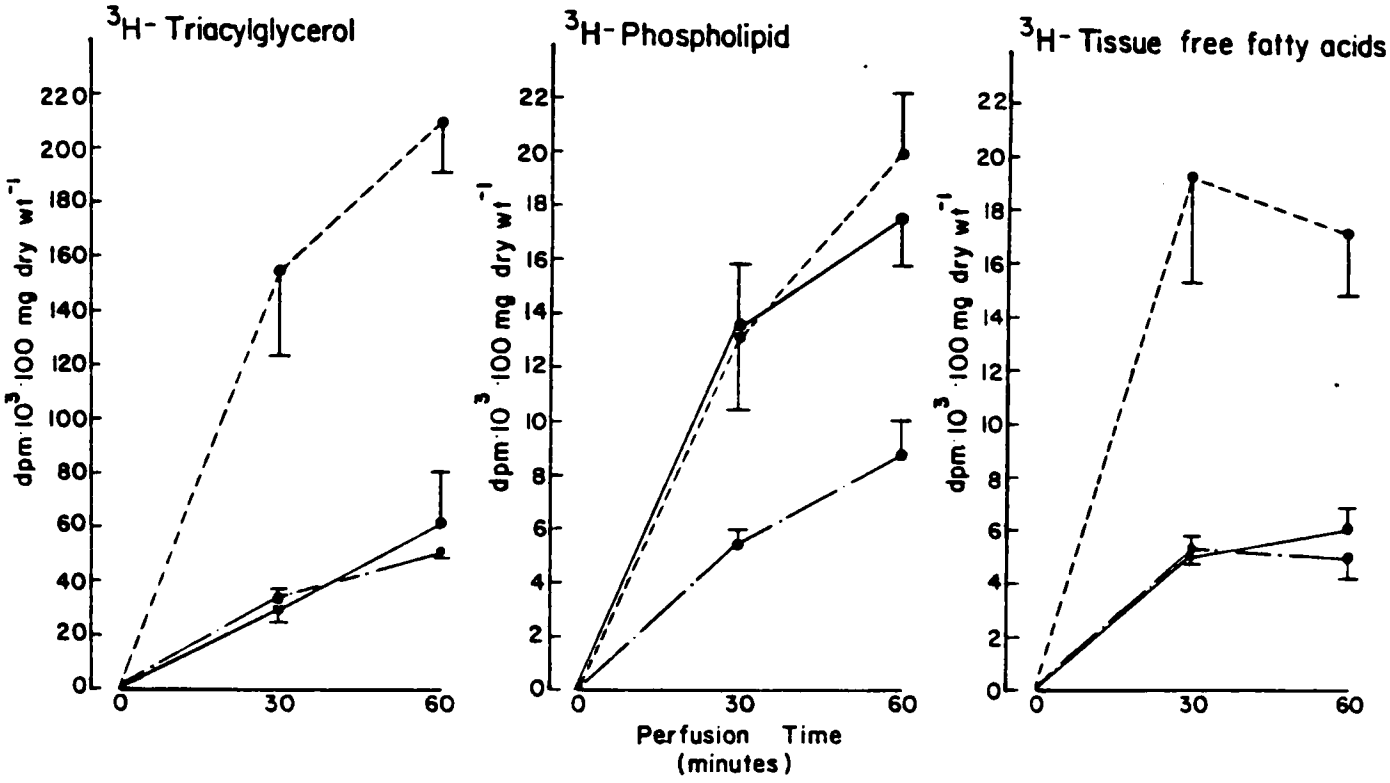

Figure 12: Incorporation of [9,10-^{3}H]-palmitate into triacylglycerol, phospholipid, and tissue free fatty acids in control and diabetic hearts. Solid lines represent control hearts perfused with normal medium, dash lines diabetic hearts perfused with diabetic medium and dash-dot lines diabetic hearts perfused with normal medium. Each point represents the mean ± S.E.

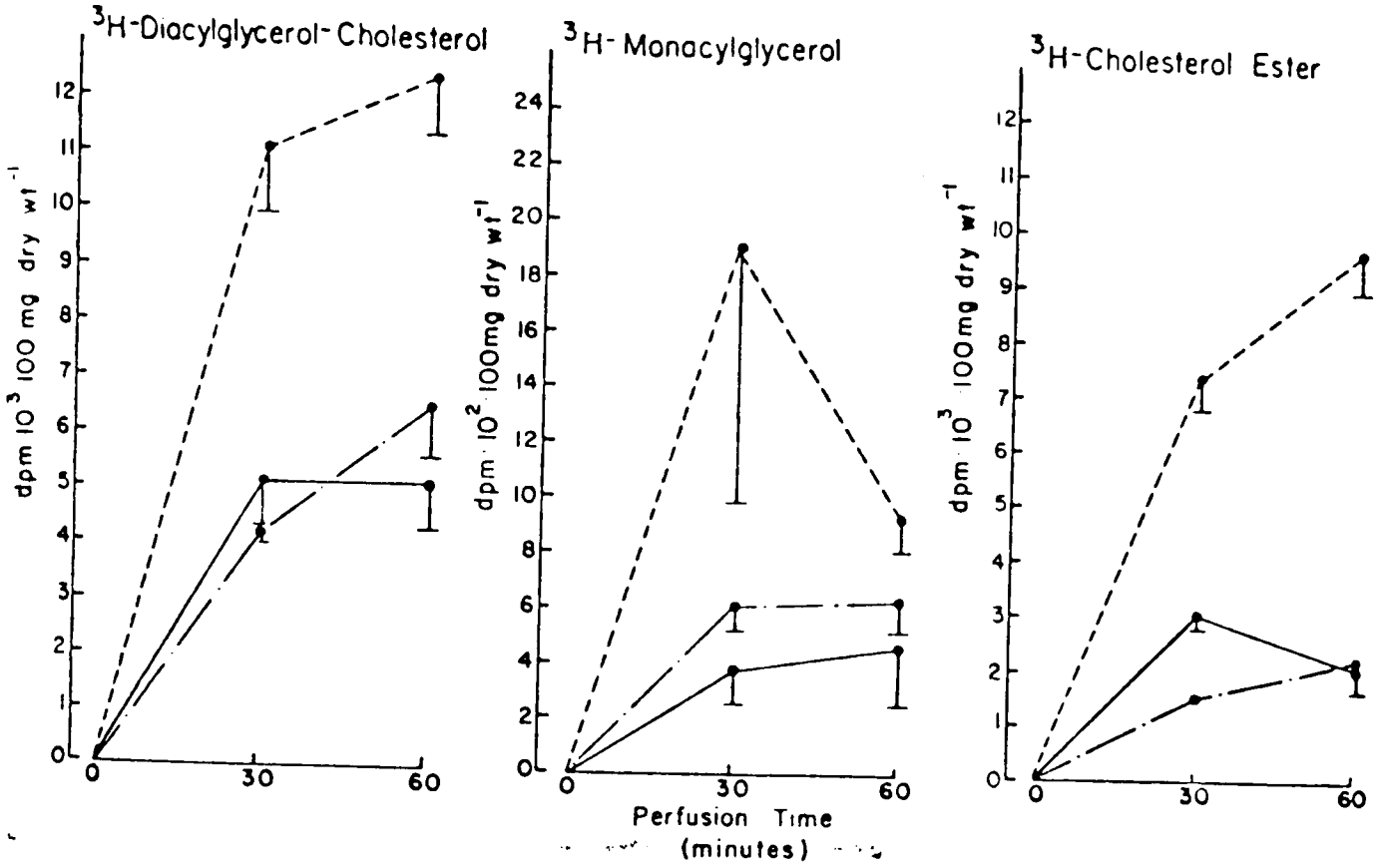

Figure 13: Incorporation of exogenous [9,10-^{3}H]-palmitate into diacylglycerol-cholesterol, monoacylglycerol and cholesterol ester in control and diabetic hearts. Solid lines represent control hearts perfused with normal medium, dash lines diabetic hearts perfused with diabetic medium and dash-dot lines diabetic hearts perfused with normal medium. Each point represents the mean ± S.E.

TABLE 13

INCORPORATION OF PERFUSATE FREE FATTY ACIDS INTO TISSUE LIPIDS OF CONTROL AND DIABETIC HEARTS AFTER 30 AND 60 MINUTES OF PERFUSION

Group	Perfusion Time (n)	Lipid Fractions						
		PL	TG	tFFA	DG-C	MG	CE	Total
Control								
	30 min (7)	0.087 ±0.013	0.199 ±0.039	0.033 ±0.002	0.033 ±0.005	0.002 ±0.001	0.020 ±0.001	0.371 ±0.053
	60 min (6)	0.111 ±0.013	0.381 ±0.104	0.037 ±0.003	0.032 ±0.004	0.002 ±0.001	0.013 ±0.002	0.577 ±0.112
Diabetic Medium								
	30 min (7)	0.088 ±0.023	1.057[a] ±0.138	0.120[a] ±0.026	0.068[a] ±0.007	0.011 ±0.005	0.045[a] ±0.003	1.365[a] ±0.170
	60 min (7)	0.018 ±0.018	1.222[a] ±0.130	0.097[a] ±0.012	0.069[a] ±0.006	0.005[a] ±0.000	0.057[a] ±0.007	1.558[a] ±0.147
Normal Medium								
	30 min (4)	0.031 ±0.004	0.197 ±0.019	0.030 ±0.002	0.024 ±0.000	0.004 ±0.001	0.008 ±0.000	0.295 ±0.019
	60 min (2)	0.048 ±0.005	0.281 ±0.009	0.028 ±0.005	0.035 ±0.006	0.003 ±0.000	0.012 ±0.000	0.409 ±0.015

NOTE: All values are expressed as μmoles/of palmitate · 100 mg dry wt^{-1} and represent the mean ± SE for the stated number of hearts (shown in parenthesis). Incorporation was calculated by dividing dpm of ^{3}H recovered in the lipid fraction (Table 7) by the medium [9,10-^{3}H]-palmitate specific activity.

[a]$P<.05$, level of significance of difference between mean values from diabetic hearts perfused with diabetic medium and control hearts perfused with normal medium.

specific activity (Table 6). Incorporation of exogenous FFA into tissue lipids was significantly ($P<0.05$) enhanced in TG, tFFA, DG-C, MG, and CE fraction but not PL of diabetic hearts perfused with diabetic medium.

CHAPTER IX

DISCUSSION

Myocardial lipid metabolism with particular attention to TG lipolysis and synthesis was investigated in perfused working hearts of control and 12-day streptozotocin-induced diabetic rats. TG lipolysis was assayed by prelabeling the heart lipids *in vivo* with palmitate-1-^{14}C followed by *in vitro* perfusion. The disappearance of ^{14}C-labeled and total TG was used as an index of lipolysis. Oxidative metabolism of endogenous lipids was determined by measuring metabolic $^{14}CO_2$ produced during perfusion. TG synthesis was estimated by the incorporation of exogenous [9, 10-^{3}H]-palmitate into cardiac TG. A unique feature of this study was that the perfusions were carried out with buffered media containing concentrations of glucose and FFA characteristic of the *in vivo* state.

In the present study, body weight, serum glucose and FFA concentrations in each experimental group were similar to the respective values obtained in Part I. In diabetes, content of myocardial TG and tFFA was increased, but PL content was similar to nondiabetic controls. These results agree with most previous reports on TG, tFFA, and PL content of the diabetic heart (20, 30, 63, 76, 77, 78). However,

one report (11) has shown a significant reduction in phosphatidylethanolamine and lysophosphatidylcholine content in the alloxan diabetic rat heart.

The prelabeling procedure involved anesthetizing a rat with ether, performing a functional hepatectomy and injection of 5 μCi palmitate-1-^{14}C-serum complex into the inferior vena cava. Thirty minutes was allowed for maximum incorporation of palmitate-1-^{14}C into heart lipids. After prelabeling, the heart was excised and perfusion-washed for 5 minutes. The distribution of radioactivity in the lipid fractions from control initial hearts was similar to previous reports (12). Diabetic rats receiving 5 μCi of palmitate-1-^{14}C had less radioactivity incorporated into tissue lipid than did control rats. Since serum FFA concentration was elevated in diabetic rats, the decreased prelabeling of heart lipids was probably due to isotopic dilution. Injection of 12 μCi of palmitate-1-^{14}C into diabetic rats significantly ($P<0.05$) increased the labeling of heart lipids. However, the apparent specific activities of TG and PL in this group were similar to those of controls. The TG fraction was preferentially labeled in both control and diabetic hearts.

Following the perfusion-washout, another group of prelabeled hearts were perfused in a recirculated working heart apparatus. Both control and diabetic hearts maintained

pressure development during the 60 minutes of perfusion. There were no group differences in systolic or diastolic pressures. Diabetic hearts showed a bradycardia; a finding which agrees with several other studies in experimental diabetes, *in vivo* (25) and *in vitro* (26). The bradycardia has been associated with a decrease in ventricular β-adrenergic receptors (79).

The rate of TG lipolysis was measured by determining the disappearance of ^{14}C-labeled TG from initial values after 30 and 60 minutes of perfusion. Control hearts were perfused with buffered media similar in substrate composition to that of normal serum, whereas diabetic hearts were perfused with media similar in substrate composition to that of diabetic serum. In addition, a group of diabetic hearts were perfused with normal media. In control hearts, there was a rapid rate of lipolysis during the first 30 minutes of perfusion as indicated by the decrease in TG radioactivity and content. During the next 30 minutes, TG lipolysis decreased. The two rates of lipolysis suggested that there may be multiple pools of TG in the myocardium; for example, one or more pools that are rapidly mobilized and depleted during the first 30 minutes of perfusion and another pool that is more slowly mobilized. Other laboratories have also suggested multiple pools of cardiac TG with different turnover rates. Stein and Stein (88)

pulse-labeled perfused hearts with [9, 10-^{3}H]-oleic acid and showed changes in the subcellular localization of the esterified fatty acid with time using radioautographic techniques. Crass et al. (13) using the prelabeling approach also obtained data which suggested the presence of multiple pools of TG. Further evidence has been obtained in hearts perfused in the absence of exogenous substrates (19, 31, 65, 83). After eventual arrest of the heart, 30-50% of TGFA remained in the tissue, indicating that only about half of the TGFA were available for energy metabolism. The stable TG pools are probably localized in lipid droplets or constitute structural components of cell membranes.

Control hearts showed a drop in TG content during perfusion even though exogenous FFA were present in normal concentrations. Obviously, the rate of TG lipolysis exceeded TG synthesis. Crass (12) has also shown an inability of the normal heart to maintain TG content when perfused with normal exogenous substrate concentrations.

Diabetic hearts perfused under diabetic substrate conditions maintained chemically-determined TG content while radioactivity decreased. This observation would suggest that the rate of TG lipolysis equalled the rate of TG synthesis. During the first 30 minutes of perfusion, lipolysis was slower in diabetic hearts perfused under diabetic conditions as compared with control hearts perfused

under normal conditions. During the next 30 minutes of perfusion, the rate of lipolysis increased slightly in diabetic hearts while in control hearts lipolysis decreased, possibly due to depletion of a rapidly mobilized TG pool. Thus, during the first 30 minutes of perfusion, lipolysis was inhibited in the diabetic myocardium relative to control hearts. This result was in conflict with the studies of Garland and Randle (30), Kreisberg (53) and Shipp et al. (83) who found enhanced rates of TG lipolysis in the 48-hour alloxan diabetic perfused rat heart. The duration of perfusion in these studies ranged from 15 to 60 minutes. The major difference between the studies of others and the present study was the concentration of exogenous substrates used during perfusion. In the above studies, hearts were perfused with or without 0.5 mM exogenous FFA. In poorly-controlled diabetes, the serum concentration of FFA is elevated (78, 80). Crass et al. (12) have shown that the addition of exogenous FFA to perfused hearts markedly inhibited TG lipolysis. Other studies (19, 31, 64) have shown that increased serum glucose concentrations such as those found in diabetes may have a slight sparing effect on myocardial TG utilization. Therefore, a possible explanation for the discrepancies between the results of the present study and those of others cited above may have been removal of inhibition of TG lipolysis by perfusion of

diabetic hearts with media a) devoid of FFA or b) containing sub-inhibitory FFA concentrations. Thus, the increased rate of lipolysis in the diabetic heart may be due to the withdrawal of inhibition in the presence of low levels of exogenous substrates. The present study tested this explanation by perfusing diabetic hearts with normal buffer. Under these conditions TG lipolysis in the diabetic myocardium was markedly enhanced. Both TG radioactivity and content decreased to control levels. Therefore, the conclusion by others (30, 53, 71, 83) that TG lipolysis in the diabetic heart was enhanced does not appear to be valid. Since diabetic heart TG content returned to control levels during perfusion with normal medium, it would be tempting to postulate that accumulation of TG in the diabetic myocardium may be localized in rapidly mobilized TG pools.

Elevated exogenous FFA inhibition of TG lipolysis may be mediated by an increase in the intracellular concentration of tFFA and/or long chain acyl CoA or acyl carnitine concentrations. Although these long chain fatty acid derivatives were not assayed in the present study, it is conceivable that these lipid intermediates may inhibit lipolysis via a type of feedback mechanism. If so, evidence presented in this study would suggest that the site of inhibition is at TG lipase. The disappearance of ^{14}C-labeled DG-C in diabetic hearts perfused with normal and diabetic

media was identical, while ^{14}C-labeled TG disappearance was slower in diabetic hearts perfused with diabetic medium. These findings suggested that TG lipase was inhibited.

The initial (pre-perfusion) tFFA content was elevated in diabetic hearts. After perfusion, content did not change in the three groups despite the observed decrease in ^{14}C-labeled tFFA. These data suggested that FFA derived from lipolysis of tissue lipids were rapidly oxidized, released into the perfusion medium or reesterified.

The data also showed that PL and CE in both control and diabetic hearts were not mobilized to any appreciable extent during perfusion. Radioactivity in these lipid fractions showed little change during perfusion. Both PL and CE are primarily structural components of cell membranes. Several other studies (13, 19, 65, 69) have also shown indirectly that PL fatty acids are probably not utilized for energy metabolism during perfusion.

Oxidation of endogenous lipid was measured by the production of $^{14}CO_2$. The production of $^{14}CO_2$ was greatest in diabetic heart perfused under normal conditions, indicating that endogenous lipid mobilization and oxidation was markedly enhanced in this group. Rates of $^{14}CO_2$ production were similar in control hearts perfused with normal medium and diabetic hearts perfused with diabetic medium. These findings suggested that the ability of diabetic heart

mitochondria to oxidize FFA produced from endogenous lipid mobilization was not impaired.

A surprising finding in the present study was the observation that ^{14}C-labeled FFA were released from the myocardium during perfusion. Diabetic hearts released more ^{14}C-labeled FFA than did control hearts. The enhanced release of ^{14}C-labeled FFA from the diabetic heart could conceivably be due to the increased intracellular content of tFFA and a greater concentration gradient.

Results from the present study also showed that the concentration of exogenous FFA in perfused working diabetic rat heart affects the amount of FFA uptake and rate of synthesis of TG. Diabetic hearts perfused under diabetic conditions had a greater FFA uptake than did normal hearts perfused under normal conditions. A greater incorporation of exogenous FFA into TG, DG, MG, and CE was associated with increased FFA uptake. Incorporation into these lipids was not increased in diabetic hearts perfused with normal medium. The incorporation of exogenous FFA into PL was not increased in diabetic hearts perfused with diabetic media as compared to control hearts. However, the incorporation into PL was greater in control hearts as compared to diabetic hearts perfused with normal media. These results suggested that TG synthesis was enhanced in diabetic hearts perfused under diabetic conditions while PL synthesis was

unchanged. Uptake of FFA by the heart is primarily dependent upon albumin:FFA ratio. Since the physiological concentration of albumin in the serum shows little variability, FFA uptake under most conditions is proportional to the extracellular concentration of FFA. In diabetes, albumin concentration decreases with duration and severity of diabetes (81). Therefore, in severe long term diabetes, the FFA-albumin molar ratio would increase. More FFA would be bound to lower affinity albumin binding sites under these conditions, thus favoring increased FFA uptake by the diabetic heart. In the present study, the increased uptake of exogenous FFA by diabetic hearts perfused with diabetic medium was due to the increased perfusate palmitate concentration. Since FFA uptake in control and diabetic hearts perfused with normal medium was similar, it appears that there were no intrinsic differences in the ability of control and diabetic hearts to take up exogenous FFA. Other reports on the uptake of exogenous FFA by diabetic heart are conflicting; i.e., unchanged (23, 76) decreased (53) or increased (33, 95).

In control and diabetic hearts perfused under normal conditions, the estimated rate of TG lipolysis exceeded the rate of TG synthesis. Thus, TG content in these two groups decreased. In contrast, the estimated rate of TG synthesis in diabetic hearts perfused under diabetic

conditions exceeded the rate of TG lipolysis. TG content would have been expected to increase. Indeed, during the first 30 minutes of perfusion diabetic heart TG content did increase slightly, but not significantly. The different rates of TG synthesis and lipolysis in this group may be due to incorporation and mobilization of FFA into and from different pools of TG. Several other studies (23, 53, 57, 63, 76) have suggested that TG synthesis in the diabetic myocardium was greatly enhanced. Increased synthesis has been shown both *in vivo* (76) and *in vitro* in the diabetic dog (57, 76) and rat heart (32, 53, 63). Factors which may contribute to an increased TG synthesis in the diabetic myocardium were discussed in Chapter V.

In summary, the results of the present study showed that TG lipolysis in the diabetic heart perfused under diabetic conditions was less than control hearts perfused under normal conditions. There were no differences in the ability of control and diabetic hearts to oxidize endogenous lipids. The results also showed that TG synthesis in diabetic hearts perfused under normal conditions was enhanced as compared to diabetic hearts perfused under diabetic conditions. Therefore, a possible mechanism is suggested for the accumulation of TG by the diabetic myocardium: the increased levels of exogenous substrates

(FFA and glucose) found in diabetic serum inhibit TG utilization while promoting TG synthesis, resulting in an accumulation of TG. These results do not support the concept of an increase TG-FFA cycle in the diabetic myocardium.

CHAPTER X

SUMMARY AND CONCLUSIONS

Two sets of experiments were performed to study possible alterations in lipid metabolism in the diabetic myocardium. The initial study, Part I, is a detailed examination of the lipid content and composition of the 12-day streptozotocin-induced diabetic rat heart. Changes in fatty acid composition of TGFA, tFFA and sFFA were found. The purpose of the second set of experiments, Part II, was to measure rates of TG synthesis, lipolysis and oxidation and determine how the level of exogenous substrates affect these rates. A summary of these results is illustrated in the schematic diagram shown in Figure 14.

In the 12-day streptozotocin-induced diabetic rat, there was a marked increase in serum glucose and FFA concentration. Associated with the increase in sFFA was a change or shift in fatty acid composition from 16-carbon fatty acids to 18-carbon fatty acids primarily stearate and linoleate. As reported in other models of experimental diabetes (18, 78), myocardial glycogen content was found to be increased in the 12-day streptozotocin-induced diabetic rat heart. Other studies have shown that the increased

Lipid Metabolism In The Diabetic Heart

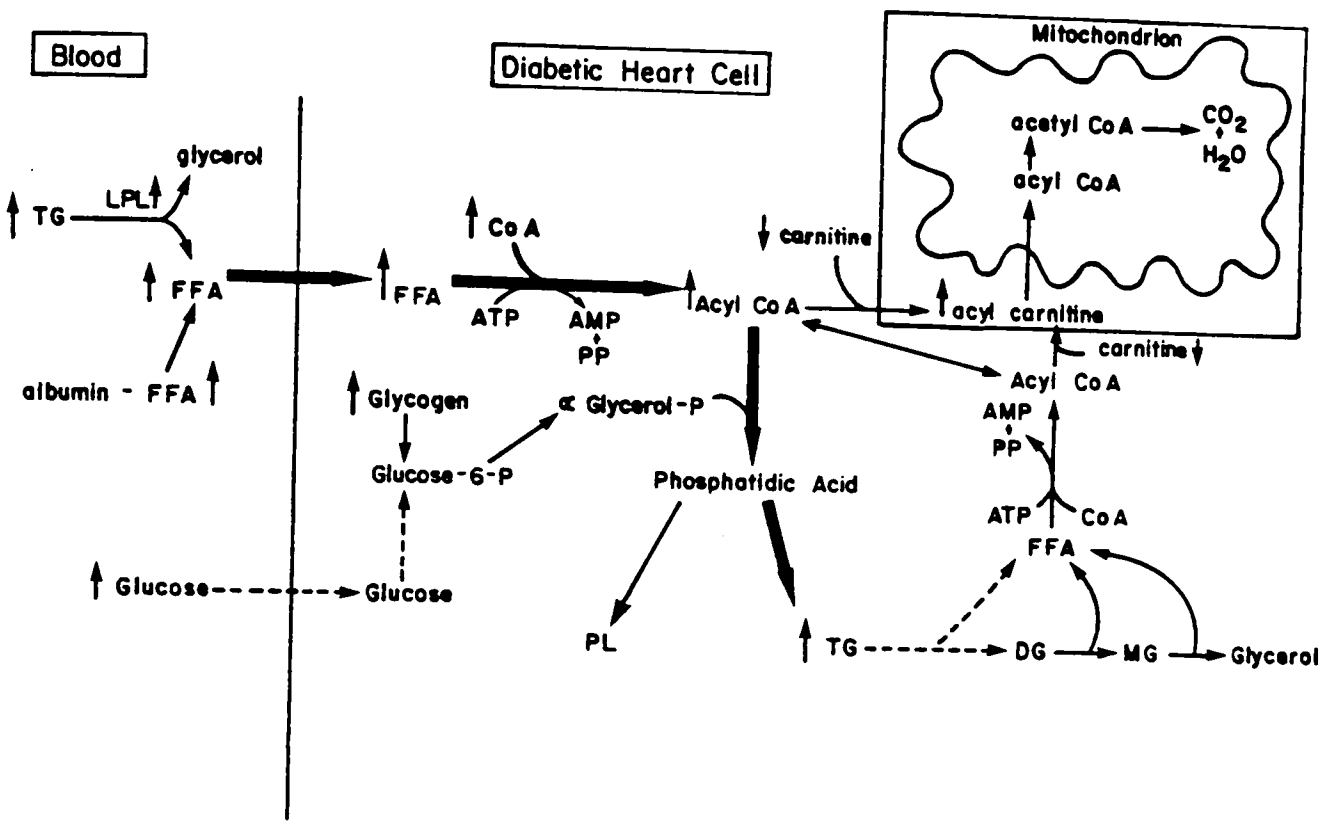

Figure 14: Lipid metabolism in the diabetic heart. Large arrow represents increased and dash arrow inhibited pathway. ↑: content is increased, ↓: content is decreased.

glycogen content was due to a decrease in glycolytic flux secondary to inhibition of the phosphofructokinase (PFK) reaction (18). This inhibition was probably due to increased intracellular citrate concentration (an inhibitor of PFK) (16), which was produced from increased fatty acid metabolism (21). Other studies have shown impaired glucose uptake and decreased glucose phosphorylation in the diabetic myocardium (29, 16, 75).

An accumulation of cardiac TG is another characteristic of experimental diabetes (20, 63, 76, 78). In the 12-day diabetic model used in this study myocardial TG content was increased up to 3-fold. Levels of tFFA were also increased. Insulin treatment of diabetic rats prevented the accumulation of TG and tFFA. These lipids accumulated despite the fact the diabetic rats were not ketoacidotic. Murthy and Shipp (63) have suggested that a marked and sustained accumulation of myocardial TG occurred only in ketotic diabetic rats. Results from the present study do not support this contention. The TGFA composition in the diabetic myocardium were similar to those of sFFA, i.e., a shift from 16-carbon fatty acids to 18-carbon fatty acids, primarily stearate and linoleate. According to the work of Evans (23), the ability of the heart to oxidize stearate and linoleate was less when compared with palmitate. Also, the incorporation of stearate

and linoleate into complex tissue lipids was greater than palmitate. Therefore, it was suggested that the described changes in sFFA and TGFA composition may be a contributing mechanism to the accumulation of TG seen in the diabetic myocardium.

PL content did not change in the 12-day streptozotocin-induced diabetic rat heart. This result was in agreement with most reports on PL content from diabetic animals (20, 78). However, one report has shown a significant reduction in PL content in the alloxan diabetic rat heart (11).

The second set of experiments, Part II, was an examination of the dynamics of lipid metabolism in normal and diabetic perfused hearts. Rates of synthesis, lipolysis and oxidation of TG in the diabetic and control hearts were determined. TG synthesis was measured by following the incorporation of exogenous [9, 10-^{3}H]-palmitate into TG. Lipolysis was evaluated by prelabeling the heart lipids *in vivo* with palmitate-1-^{14}C and following the disappearance of ^{14}C-labeled TG during 30 and 60 minutes of *in vitro* perfusion. Endogenous lipid oxidation was determined by quantitative assessment of metabolic $^{14}CO_2$ produced during perfusion. Control and diabetic hearts were perfused with a bicarbonate buffer containing the concentrations of glucose and FFA characteristic of serum values *in vivo*.

In diabetic hearts perfused under diabetic conditions, FFA uptake and rate of TG synthesis were greater than normal hearts perfused under normal conditions. Enhanced synthesis of TG in the diabetic heart has been shown by a number of studies (23, 53, 57, 63, 76). An interesting finding of this study was that TG synthesis was only enhanced in the diabetic heart when the level of exogenous substrates were at diabetic concentrations.

TG lipolysis in diabetic hearts perfused under diabetic conditions was less than control hearts perfused under normal conditions. The results contradict the findings of several other reports (20, 30, 53, 82). Much of this previous work reported enhanced lipolysis in the alloxan diabetic rat heart. The discrepancy between these studies and the present study was shown to be due to the level of exogenous substrates used during perfusion. For example, perfusion of diabetic hearts in the presence or absence of 0.5 mM exogenous FFA resulted in enhanced rates of lipolysis. In the present study when diabetic hearts were perfused under normal conditions, lipolysis was also markedly enhanced. We concluded that the high levels of exogenous substrates found in diabetic serum inhibited TG lipolysis. The point of inhibition is suggested to be at TG lipase. Therefore, a possible mechanism is suggested for the accumulation of TG by the diabetic myocardium: the

increased levels of exogenous substrates, FFA and glucose, found in diabetic serum inhibit TG utilization while promoting TG synthesis; thus resulting in an accumulation of TG. These results do not support the concept of an increased TG-FFA cycle in the diabetic myocardium.

In the present study, no differences were observed in $^{14}CO_2$ production between control and diabetic hearts perfused under in vivo conditions. Diabetic hearts perfused under normal conditions showed a marked increase in endogenous lipid oxidation. These results suggest that there were no deficiencies in the ability of diabetic mitochondria to take up and oxidize cytosolic FFA produced from endogenous lipid mobilization. Oxidation of exogenous FFA was not measured in this study.

There are also a number of metabolic alterations of the diabetic heart that were not measured in the present study; some of these are included in the schematic of lipid metabolism in the diabetic heart (Figure 14). Other lipid abnormalities reportedly found in diabetes are a) decreased cardiac carnitine content (6, 24), b) decreased total body carnitine and increased turnover (59) c) increased cardiac Coenzyme A content (24), d) increased cardiac long chain acyl CoA and acyl carnitine (6, 24, 30), e) increased (22, 49) or decreased (1, 53, 60) myocardial lipoprotein lipase activity, f) increased flow of glucose carbon atoms through

α-glycerol phosphate to TG despite inhibition of glucose uptake and glycolysis (21), and g) increased serum TG concentration.

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