Basic Research in
Cardiology
Basic Res Cardiol 84:165–173 (1989)

# Enhanced lipolysis of myocardial triglycerides during low-flow ischemia and anoxia in the isolated rat heart

K. Schoonderwoerd, S. Broekhoven-Schokker, W. C. Hülsmann, and H. Stam

Department of Biochemistry I, Medical Faculty, Erasmus University Rotterdam, Rotterdam, The Netherlands

*Summary:* We studied lipolysis in the isolated rat heart, measured as glycerol release during anoxia, low-flow ischemia and subsequent reperfusion. It was found that the rate of lipolysis was enhanced during ischemia/anoxia while the lipase activities in tissue extracts involved in the myocardial lipolysis and the amount of triglycerides were not affected. This indicates the dominant occurrence of a lipolysis-reesterification principle in ischemic and anoxic tissue. A common observation of ischemia/anoxia is an increase in the tissue NADH/$NAD^+$ ratio. Therefore we investigated the effect of lactate and malate, both of which enhance the tissue redox state on myocardial lipolysis. Perfusion in the presence of lactate (10 mM) and malate (10 mM) both stimulated myocardial lipolysis by about five times. This suggests that the rate of reesterification of product fatty acids to triglycerides, which is determined by the NADH/$NAD^+$ ratio, because of the increased formation of glycerol 3-phosphate from dihydroxy acetone phosphate, plays an important role in the regulation of lipolysis. The existence of triglyceride-fatty acid-triglyceride cycle is discussed.

*Key words:* Ischemia; anoxia; myocardial lipolysis; reesterification; free fatty acids; redox state

## Introduction

In the normoxic heart fatty acids derived from endogenous triglycerides are an important source of energy. More than 60 % of the total oxygen consumption is accounted for by the beta-oxidation of free fatty acids (21, 23). During ischemia and anoxia, however, when beta-oxidation is hampered, glucose is the preferred substrate (22, 24). Much is known about the mechanism by which the rates of glycogenolysis and glycolysis are affected under both conditions of oxygen deprivation (11, 18, 25, 30). During ischemia glyceraldehyde-3-phosphate dehydrogenase becomes the rate-limiting enzyme in glycolysis whereas in the anoxic myocardium pyruvate dehydrogenase was shown to be the rate-limiting enzyme (25, 30). By contrast, still a large controversy exists about the fate of myocardial triglycerides under ischemic and anoxic circumstances. Both an inhibition of lipolysis (4) and a stimulation of triglyceride synthesis (31), as well as a stimulation of lipolysis (9, 25, 41) and an inhibition of triglyceride synthesis have been described during ischemia/anoxia. A common feature of myocardial ischemia and anoxia is the rapid increase in the intracellular NADH/$NAD^+$ ratio, inhibiting the beta-oxidation of intracellular fatty acids (20, 30, 43). As a consequence of the elevated redox state of the tissue and the subsequent inhibition of the beta-oxidation, there is an enhanced production of glycerol-3-phosphate from dihydroxy acetone phosphate and long chain fatty acyl-CoA, both substrates for the synthesis of triglycerides. Such conditions result in an enhanced reesterification of fatty acids to form triglycerides. A triglyceride-fatty acid energy wasting cycle has been proposed during

ischemia/anoxia (25, 28, 41). Increased levels of fatty acids may be harmful for the heart because of their inhibitory effect on the adenine nucleotide translocase in mitochondria (35), $Na^+,K^+$-ATPase in the sarcolemma (14) and calcium pump in the sarcoplasmatic reticulum (13). Therefore knowledge about the fate of the liberated fatty acids and the occurrence of an ATP wasting triglyceride-free fatty acid cycle in (ischemic and anoxic) heart tissue is of great importance.

## Materials and methods

### *Animals*

Male Wistar rats (200–300 g) were used throughout the study. They were kept under an artificial light cycle of 12 h (7–19 hours) and had free access to control laboratory chow and water until the beginning of the experiment.

### *Perfusion protocol*

Under light ether anesthesia the heart was quickly excised and perfused retrogradely as described elsewhere (37). After a 30 min preperfusion, low-flow ischemia was induced by reduction of the flow to 1 ml/min using a peristaltic pump. Anoxia was introduced by perfusion with buffer equilibrated with 95 % $N_2$:5 % $CO_2$ during 30 min whereafter the hearts were reperfused with control medium. At different time intervals the effluent samples were collected for measuring glycerol release. At the end of the experiments the hearts were removed from the apparatus and homogenized in 0.25 M sucrose, 10 mM Tris/HCl, 1 mM EDTA, pH 7.4 (38). In some experiments the hearts were perfused with $^{14}$C-glucose (1.66 mCi/mmol) during the ischemic period. At the end of this period the hearts were quickly frozen and tissue lipids were extracted with chloroform/methanol (2:1) and separated on silicic acid plates, developed with heptane/diethyl ether/acetic acid (60:40:1, v/v). Triglycerides were scraped from the plates and counted with Instagel in a liquid scintillation counter.

### *Biochemical assays*

TG lipase activity was measured using a [$^3$H]-triolein/gum acacia emulsion as previously described (34, 38). Protein content was determined by the biuret method (8), using bovine serum albumin as standard. Glycerol in effluent samples was determined by the method of Laurell and Tibbling (15). Glycerol-3-phosphate acyltransferase (G-3-PAT) was measured based on the method of Yamashita and Numa (44). We used a reaction mixture of the following composition: 35 mM Tris/HCl buffer pH 7.4, 1 mM glycerol-3-phosphate and 60 µmol palmitoyl-CoA which was incubated at 30 °C. Triglyceride was estimated by the method of Laurell (16). The results are presented as mean values ± standard error of the mean.

*Reagents* were usually from Merck (Darmstadt, FRG). Bovine serum albumin, triacylglycerol, palmitoyl-CoA, lactate, glycerol-3-phosphate were from Sigma (St. Louis, M.O., U.S.A.), NADH, ATP, $NAD^+$, glycerokinase, glycerol-3-phosphate dehydrogenase, were purchased from Boehringer (Mannheim, FRG). Cysteine-hydrochloride was from B.D.H. Chemicals (Poole, UK) and [$^3$H]-triolein and L-U-$^{14}$C glycerol-3-phosphate were from Amersham International PLC (Amersham, UK).

### *Statistical analysis*

Student's *t*-test was used to determine the significance of the differences.

## Results

Lipolysis was studied in ischemic and anoxic rat hearts. As presented in Figure 1 glycerol release was stimulated within 5 min after the onset of low-flow ischemia and reached a plateau phase after 10 min. A burst in the release of glycerol was observed at the start of the free reperfusion period (constant pressure 80 cm $H_2O$) probably representing release of

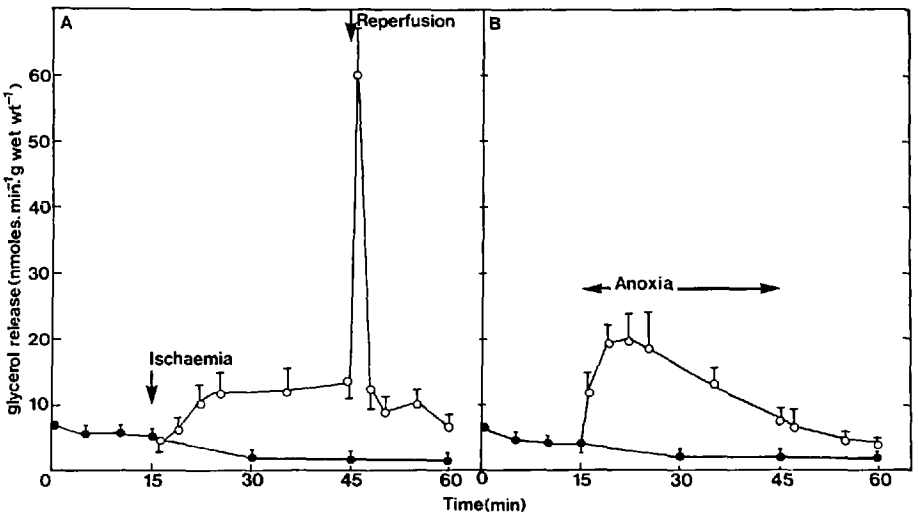


Fig. 1. The effect of low-flow ischemia and anoxia upon myocardial lipolysis as measured as glycerol release. The results represent the mean value ± SEM of 4–6 separate experiments. ●, glycerol release during normoxic perfusion. ○, glycerol release during ischemia/reperfusion (A) and anoxia/reoxygenation (B).

glycerol formed during the previous ischemic period. During initial anoxia glycerol release was markedly enhanced, reaching its maximum after 5–7 min, followed by a gradual decline to control levels thereafter. To study whether an increased neutral lipase or acid lipase activity was responsible for the enhanced rate of lipolysis during low-flow ischemia we estimated these lipase activities in heart homogenates prepared after the ischemic period. Neither the neutral lipase (control 0.63 ± 0.05, ischemia 0.62 ± 0.03 mU/g wet weight) nor

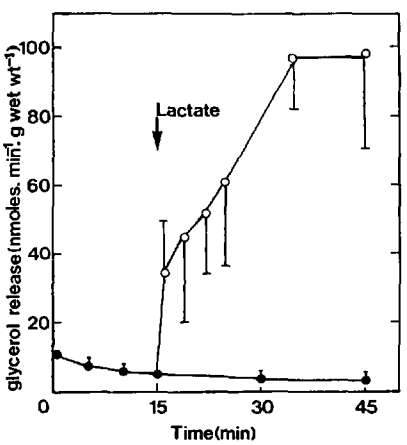


Fig. 2. Effect of lactate (10 mM) upon glycerol release from isolated perfused rat hearts. The data are the mean ± SEM of four experiments. ●, control; ○, 10 mM lactate.

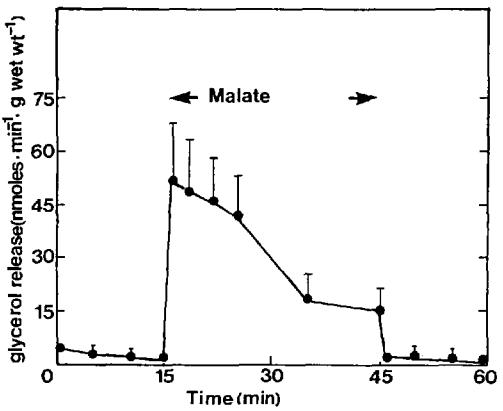


Fig. 3. Effect of malate (10 mM) upon glycerol release from isolated perfused rat hearts. The results represent the mean value ± SEM of four separate experiments. ●, control; ○, 10 mM malate.

the acid lipase activity (control 0.53 ± 0.03, ischemia 0.54 ± 0.06 mU/g wet weight) was altered in tissue samples after low-flow ischemia.

A common feature of myocardial ischemia and anoxia is the rapid increase in the intracellular NADH/$NAD^+$ ratio. To study the effect of increased NADH/$NAD^+$ ratios on myocardial lipolysis, we perfused rat heart in the presence of 10 mM lactate and 10 mM malate. It is generally accepted that both measures lead to an increase in the tissue NADH/$NAD^+$ ratio, lactate perfusion as a consequence of its conversion to pyruvate, malate perfusion due to the malate-aspartate shuttle. Perfusion in the presence of lactate produced a transient increase of glycerol release which was maximal after 20 min (Fig. 2). Addition of malate to the perfusion buffer gave rise to a fast increase in glycerol release from the heart within 1 min. After a plateau phase of about 10 min, lipolysis gradually decreased during the following 20 min (Fig. 3). Subsequent perfusion of the heart with control buffer was rapidly followed by a fall in lipolysis to preperfusion levels. To study whether the increased rate of lipolysis was the consequence of the increased supply of substrates, the effects of glucose-free and pyruvate perfusion on glycerol release were measured (Fig. 4). Perfusion in the

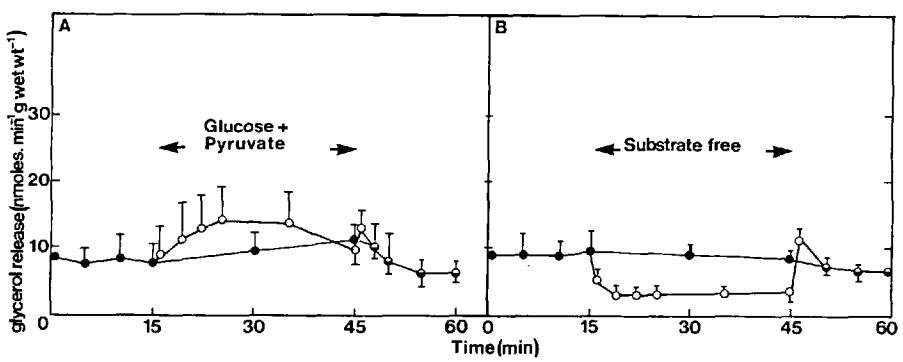


Fig. 4. Effect of pyruvate (10 mM) (A) and substrate-free perfusion (B) upon glycerol release from isolated perfused rat hearts. The results represent the mean value ± SEM of four experiments. ●, control; ○, 10 mM pyruvate (A) and substrate-free perfusion (B).

Table 1. The effect of low-flow ischemia, anoxia and malate perfusion on myocardial triglyceride content.

| | n | TG (μg/g wet weight) |
|---|---|---|
| Control | 6 | 620 ± 160 |
| Ischemia | 6 | 710 ± 83 |
| Anoxia | 6 | 422 ± 116 |
| Malate (10 mM) | 3 | 1035 ± 86 |

Triglycerides were measured in rat heart homogenates prepared 30 min after the onset of ischemia, anoxia or malate perfusion.

presence of 10 mM pyruvate increased the rate of glycerol release only slightly – probably by a better washout of glycerol from the tissue as a consequence of an increase in the flow observed during perfusion with pyruvate. Omission of glucose in the perfusion buffer after a 15 min preperfusion on the other hand reduced lipolysis.

As demonstrated earlier, myocardial lipolysis is regulated by product inhibition (5, 34, 36, 39). During low-flow ischemia and anoxia beta-oxidation is hampered and reesterification of endogenous fatty acids to triglycerides is the only way to remove free fatty acids from the site of lipase action. In vitro measurements of glycerol-3-phosphate acyltransferase (G-3-PAT) activity (9) have indicated rapid alterations of G-3-PAT activity during myocardial ischemia. Therefore, we measured the activity of G-3-PAT the rate limiting enzyme on the triglyceride synthesis (1), in homogenates of post-ischemic or post-anoxic tissue. G-3-PAT activity was not changed neither after ischemia nor after anoxia (control 34.1 ± 2.9, ischemia 35.5 ± 1.0 nmoles/min/g wet weight).

Recently the existence of a triglyceride-fatty acid-triglyceride cycle in heart tissue was proposed (28). To investigate whether a triglyceride-fatty acid cycle would be operative in rat heart, we estimated the amount of triglycerides during ischemia/anoxia and malate perfusion. From Table 1 it can be concluded that no net decrease in tissue triglycerides was found during ischemia and malate perfusion. To determine the incorporation of glycerol-3-phosphate, rat hearts were perfused with $^{14}$C-glucose during the ischemic period. After perfusion the hearts were quickly frozen and tissue lipids were extracted and analyzed.

It was found that during the ischemic period 20 ± 5 (n = 3) nmoles glucose were incorporated in the lipid fraction indicating that reesterification occurs. The estimated incorporation of glycerol-3-phosphate accounts only for 10 % of the incorporation, calculated from the rate of lipolysis, which may be explained by enhanced glycogenolysis observed after ischemia.

## Discussion

In this paper we present evidence for an important role of the tissue NADH/NAD$^+$ ratio in the regulation of lipolysis. Lipolysis was enhanced during low-flow ischemia and anoxia. This finding is in accordance with the results of Vik-Mo et al. (41) in dog heart, Heathers and Brunt (9) and Trach et al. (40) in rat heart but at variance with data from Crass III and Pieper (4). However, we did confirm the results of Crass III et al. (4) who also did not find a decrease in myocardial triglyceride content during perfusion of rat hearts, prelabeled with [1-$^{14}$C]palmitate with a hypoxic perfusion medium equilibrated with 5 % $O_2$. The mechanism underlying the enhanced rate of lipolysis during low-flow ischemia and anoxia has not yet been revealed. A number of possibilities have been proposed recently. For instance, the presence of a hormone sensitive triglyceride lipase (3, 7, 9, 17, 26) activated by an increase

in cytosolic cAMP or $Ca^{2+}$ levels as a consequence of the endogenous release of catecholamines occurring during ischemia or anoxia (9, 19, 32). However, in our opinion the evidence for the existence of a hormone-sensitive lipase is not convincing since the observed activation of neutral lipase activity by cAMP was due to an enhanced glycogenolysis (33). Furthermore the activation of a hormone-sensitive lipase does not play a role in enhanced lipolysis during ischemia/anoxia because there was no change in lipolysis using different $Ca^{2+}$ concentrations during anoxia (results not shown). In addition Dart et al. (6) did not find a change in noradrenaline release from glucose-perfused hearts during ischemia.

It has been suggested before that lipolysis in heart may mainly be regulated by product inhibition (5, 34, 36, 39). In heart there are several ways to remove the inhibitory free fatty acids (Fig. 5); beta-oxidation, reesterification and transport from the site of formation. We propose that the withdrawal of inhibitory free fatty acids would mainly be determined by the rate of reesterification, which is regulated by the tissue $NADH/NAD^+$ ratio. Recently we have shown that the observed activation of neutral lipase activity by cAMP was due to enhanced glycogenolysis, delivering glycerol-3-phosphate for reesterification of the inhibitory free fatty acids (33). During ischemia and anoxia beta-oxidation is hampered and the rates of glycogenolysis and glycolysis are enhanced. Glyceraldehyde-3-P dehydrogenase, catalyzing the conversion of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate, is inhibited by the increased $NADH/NAD^+$ ratio. Therefore a large amount of glycerol-3-phosphate is formed from dihydroxyacetone phosphate and reesterification becomes much more relevant for the withdrawal of inhibitory free fatty acids. Because the endogenous level of glycerol-3-phosphate exceeds the amount of intracellular free fatty acids (1 mmol and 0.1 μmol/g wet weight, respectively) formed by lipolysis of endogenous triglycerides, net triglyceride synthesis will occur with exogenous free fatty acids taken up from the circulation. This could be an explanation for the lipid accumulation observed after ischemia.

This accumulation is mainly found in the borderline ischemic tissue with no or little change in the central ischemic region (12). Because of the severe reduction of coronary flow in the central ischemic region, not only the deliverance of exogenous fatty acids is reduced but also the availability of glucose. In the borderline tissue residual flow will make possible the supply of exogenous fatty acids and glucose, glycogenolysis and ATP synthesis. This allows for the reesterification and subsequent triglyceride accumulation (25, 30, 40). Moreover, the lipid accumulation in ischemic cat heart was reduced by β-adrenergic antagonists (12), which may be a consequence of a reduced concentration of circulating fatty acids as well as an inhibition of heart cell glycogenolysis. These results confirm the previous suggestions of a triglyceride-free fatty acid cycle operating during ischemia and anoxia (28, Fig. 5). Such a cycle is a wasteful, ATP consuming, cycle. It can be calculated from the glycerol release of the ischemic heart (15 nmoles/min/g wet weight) and the unchanged level of triglycerides after the perfusion that 15 nmoles glycerol-3-phosphate must be formed from glycogenolysis and/or glycolysis for reesterification. Generation of glycerol-3-phosphate from glycogenolysis will cost 7.5 nmoles ATP/min/g wet weight. Activation of free fatty acids generated from lipolysis of endogenous triglycerides will cost 90 nmoles ATP/min/g wet weight. Assuming ATP can only be formed from glycogenolysis and glycolysis, and a lactate production of 2 μmoles/min/g wet weight, respectively, 3 or 2 μmoles ATP/min/g wet weight are generated, indicating an energy loss of only 3.3–4.4 % for the ischemic heart. This is in agreement with the results of Trach et al. (40). Taken into consideration that 45 nmoles free fatty acids are reesterified in this process, bearing a potential of about 6500 nmoles ATP, it can be calculated that the energy investment for reesterification of fatty acids is only 1.5 % of the total energy production after β-oxidation. Therefore accumulation of free fatty acids during ischemia will occur only after a fall of the ATP level (27) or after exhaustion of glycogen, the precursor of glycerol-3-phosphate. Despite the enhanced rate of lipolysis during lactate and malate

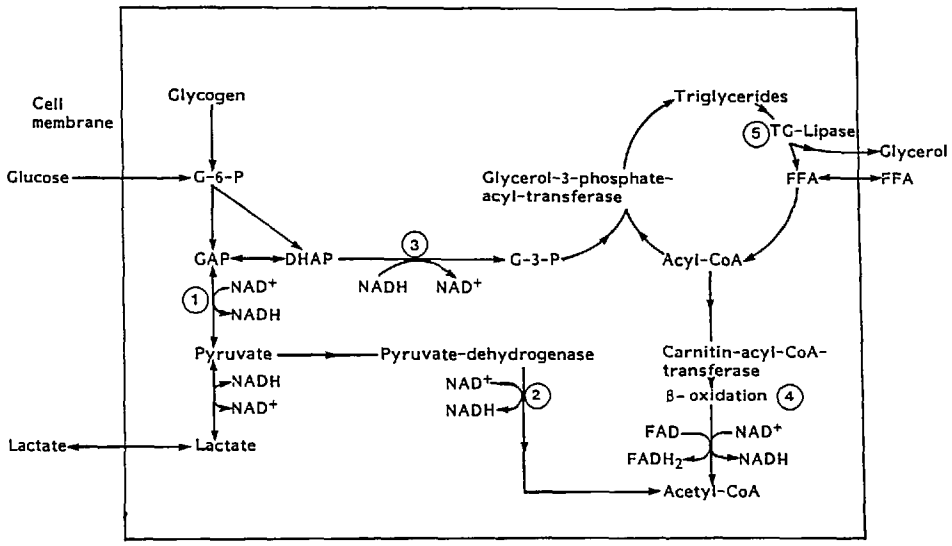


Fig. 5. Schematic representation of the effects of the tissue NADH/$NAD^+$ ratio on myocardial glycolysis and lipolysis. Proposed triglyceride-free fatty acid cycle. 1) Inhibition of the conversion of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate by an enhanced NADH/$NAD^+$ ratio; 2) Inhibition of pyruvate dehydrogenase by an increased NADH/$NAD^+$ ratio and acetyl-CoA; 3) Stimulation of the formation of glycerol-3-phosphate (G-3-P); 4) Inhibition of beta-oxidation by an increased redox state; 5) Inhibition of TG-lipase by free fatty acids.

perfusion no decrease in the amount of triglycerides was found (Table 1). This could be a consequence of the enhanced formation of glycerol-3-phosphate because of a higher NADH/$NAD^+$ ratio and subsequent reesterification of the liberated free fatty acid with glycerol-3-phosphate. An increased incorporation of free fatty acids in endogenous lipids has been found during simultaneous infusion with lactate (2, 42). Therefore it can be concluded that also in the normoxic heart the triglyceride-free fatty acid cycle is operative. This leads to the question whether free fatty acids are esterified before subsequent lipolysis and beta-oxidation. Such a mechanism was first proposed by Zierler (45) in human forearm muscle. Also our study supports such a mechanism. Further studies are necessary to solve this question.

In conclusion our results confirm the tight coupling between glycogenolysis, glycolysis, and lipolysis in the normoxic (10, 29) as well as in the ischemic and anoxic heart (40). This triglyceride-free fatty acid cycle, although ATP consuming, may be very important for the protection of the heart tissue against the deleterious effects of elevated levels of intracellular free fatty acids.

*Acknowledgements*

The authors thank Miss Cecile Hanson for the preparation of the manuscript.

*References*

1. Bell DR, Coleman RA (1983) Enzymes of triacylglycerol formation in mammals. In: Boyer PD (ed) The Enzymes. Academic Press Inc Vol XVI, pp 87–111
2. Bielefeld DR, Vary TC, Neely JR (1985) Inhibition of carnitine palmitoyl-CoA transferase activity and fatty acid oxidation by lactate and oxfenicine in cardiac muscle. J Mol Cell Cardiol 17:619–625

3. Christian DR, Kilsheimer GS, Pettet G, Paradise P, Ashmore J (1969) Regulation of lipolysis in cardiac muscle: A system similar to the hormone sensitive lipase of adipose tissue. Adv Enzyme Regul 7:71–83
4. Crass III MF, Pieper GM (1975) Lipid and glycogen metabolism in the hypoxic heart. Effects of epinephrine. Am J Physiol 229:885–890
5. Crass III MF (1977) Regulation of triglyceride metabolism on the isotopically prelabeled perfused heart. Fed Proc 36:1995–1999
6. Dart AM, Riemersma RA, Schömig A, Ungar A (1987) Metabolic requirements for release of endogenous noradrenaline during myocardial ischaemia and anoxia. Br J Pharmacol 90:43–50
7. Goldberg DI, Khoo JC (1985) Activation of myocardial neutral triglyceride lipase and neutral cholesteryl esterase by cAMP-dependent protein kinase. J Biol Chem 260:5879–5882
8. Gornall AG, Bardawill CJ, David MM (1949) Determination of serum proteins by means of the biuret reaction. J Biol Chem 177:751–766
9. Heathers GP, Brunt RV (1985) The effect of coronary artery occlusion and reperfusion on the activities of triglyceride lipase and glycerol-3-phosphate acyltransferase in the isolated perfused rat heart. J Mol Cell Cardiol 17:907–916
10. Hülsmann WC, Stam H (1979) Lipolysis in heart and adipose tissue, effects of inhibition of glycogenolysis and uncoupling of oxidative phosphorylation. Biochem Biophys Res Commun 88:867–872
11. Ichihara K, Abiko Y (1982) Cross-over plot study of glycolytic intermediates in the ischemic canine heart. Jap Heart J 23:817–828
12. Jodalen H, Stangeland L, Grong K, Vik-Mo H, Lekven J (1985) Lipid accumulation in the myocardium during acute regional ischaemia in cats. J Mol Cell Cardiol 17:973–980
13. Katz AM, Nash-Adler P, Miceli J, Messineo F, Louis CF (1979) Inhibition of Ca-efflux from the sarcoplasmatic reticulum by free fatty acids. Circulation 59:11–12
14. Lamers JMJ, Hülsmann WC (1977) Inhibition of $(Na^+ + K^+)$-stimulated ATPase of heart by fatty acids. J Mol Cell Cardiol 9:343–346
15. Laurell S, Tibbling G (1966) An enzymatic fluorometric micromethod for the determination of glycerol. Clin Chim Acta 13:317
16. Laurell A (1966) A method for routine determination of plasma triglycerides. Scand J Clin Lab Invest 18:668–672
17. Lech JJ, Jesmok GJ, Calvert DN (1977) Effects of drugs and hormones on lipolysis in heart. Fed Proc 36:2000–2008
18. Liedtke AJ (1981) Alterations of carbohydrate and lipid metabolism in the acutely ischemic heart. Prog Cardiovasc Dis 23:321–337
19. Malliani A, Schwartz PJ, Zanchetti A (1969) A sympathetic reflex elicited by experimental coronary occlusion. Am J Physiol 217:703–709
20. Moore KH (1985) Fatty acid oxidation in ischemic heart. Molec Physiol 8:549–563
21. Neely JR, Rovetto MJ, Oram JF (1972) Myocardial utilization of carbohydrate and lipids. Prog Cardiovasc Dis 15:289–329
22. Neely JR, Morgan HE (1974) Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Ann Rev Physiol 36:413–459
23. Olson RE, Hoeschen RJ (1964) Utilization of endogenous lipid by the isolated perfused rat heart. Biochem J 103:796–801
24. Opie LH, Owen P, Riemersma RA (1973) Relative rates of oxidation of glucose and free fatty acids by ischemic and non-ischemic myocardium after coronary artery ligation in the dog. Eur J Clin Invest 3:419–435
25. Opie LH (1976) Metabolic regulation in ischemia and hypoxia II. Effects of regional ischemia on metabolism of glucose and fatty acids. Relative rates of aerobic and anaerobic energy production during myocardial infarction and comparison with effects on anoxia. Circ Res 38 Suppl I:52–68
26. Palmer WK, Caruso RA, Oscai LB (1987) Protein kinase inhibitor blocks the activation of a myocardial triacylglycerol lipase. J Mol Cell Cardiol 19:653–659
27. Prinzen FW, Van der Vusse GJ, Artz T, Roemen THM, Caumans WH, Reneman RS (1984) Accumulation of non-esterified fatty acids in ischemic canine myocardium. Am J Physiol 247:H264–H272

28. Riemersma RA (1987) Raised plasma non-esterified fatty acids (NEFA) during ischaemia: implications for arrhythmias. Basic Res Cardiol 82 Suppl 1:177–186
29. Rösen P, Budde Th, Reinauer H (1981) Triglyceride lipase activity in the diabetic rat heart. J Mol Cell Cardiol 13:539–550
30. Rovetto MJ, Lamberton WF, Neely JR (1975) Mechanism of glycolytic inhibition in ischemic rat hearts. Circ Res 37:742–752
31. Scheuer J, Brachfeld N (1966) Myocardial uptake and fractional distribution of palmitate-1-$^{14}$C by the ischemic dog heart. Metabolism 15:945–954
32. Schömig A, Dart AM, Dietz R, Mayer E, Kübler W (1984) Release of endogenous catecholamines in the ischemic myocardium of the rat. Part A; Locally mediated release. Circulation Res 41:606–615
33. Schoonderwoerd K, Broekhoven-Schokker S, Hülsmann WC, Stam H (1987) Stimulation of myocardial neutral triglyceride lipase activity by adenosine-3':5'-monophosphate: involvement of glycogenolysis. Basic Res Cardiol 82 Suppl 1:29–36
34. Severson DL, Hurley B (1982) Regulation of rat heart triacylglycerol ester hydrolases by free fatty acyl-CoA and fatty acylcarnitine. J Mol Cell Cardiol 14:467–474
35. Shug A, Shrago E, Bittar N, Folts JD, Koke JR (1975) Acyl-CoA inhibition of adenine nucleotide translocation in ischemic myocardium. Am J Physiol 228:689–692
36. Stam H, Geelhoed-Mieras MM, Hülsmann WC (1980) Erucic acid-induced alteration of cardiac triglyceride hydrolysis. Lipids 15:242–250
37. Stam H, Hülsmann WC (1982) Effects of hormones, amino acids and specific inhibitors on rat heart heparin releasable lipoprotein lipase and tissue lipase activities during long term perfusion. Biochim Biophys Acta 794:72–82
38. Stam H, Broekhoven-Schokker S, Hülsmann WC (1985) Characterization of mono-, di- and triacylglycerol lipase activities in the isolated rat heart. Biochim Biophys Acta 875:76–86
39. Stam H, Hülsmann WC (1985) Regulation of lipases involved in the supply of substrate fatty acids for the heart. Eur Heart J 6:158–167
40. Trach V, Buschmans-Denkel E, Schaper W (1986) Relationship between lipolysis and glycolysis during ischemia in the isolated rat heart. Basic Res Cardiol 81:454–464
41. Vik-Mo H, Riemersma RA, Mjøs OD, Oliver MF (1979) Effect of myocardial ischemia and antilipolytic agents on lipolysis and fatty acid metabolism in the in situ dog heart. Scand J Clin Lab Invest 39:559–568
42. Visser FC, Van Eenige MJ, Duwel CMB, Roos JP (1986) Radioiodinated free fatty acids; can we measure myocardial metabolism? Eur J Nucl Med 12:S20–S23
43. Whitmer JT, Idell-Wenger JA, Rovetto MJ, Neely JR (1977) Control of fatty acid metabolism in ischemic and hypoxic hearts. J Biol Chem 253:4305–4309
44. Yamashita S, Numa S (1981) In: Lowenstein JM, Colowick SP, Kaplan NO (eds) Methods in Enzymology. Lipids Part C. Volume 71. Academic Press, New York, pp 550–555
45. Zierler KL (1976) Fatty acids as substrates for heart and skeletal muscle. Circulation Res 38:459–463

Received November 9, 1987

Authors' address:

K. Schoonderwoerd, Department of Biochemistry I, Medical Faculty, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands