H. Stam / G. J. van der Vusse (eds.)

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Preface

Scientific progress with respect to the important role of various lipids in myocardial function and the dearrangements of lipid metabolism underlying ischaemic heart disease(s) has been considerable in recent years. In 1986 alone, an overall number of 1044 full papers covering the topics "Lipids-Heart-Heart Disease", have been published in a variety of biochemistry, physiology, pharmacology and cardiology journals (source: Index Medicus). They have broadened our insight into the molecular basis of myocardial lipidology and the lipido-chemical basis of cardiology in health and disease, and have narrowed the gap between promising pharmacological intervention in experimental animals and clinical treatment of patients suffering from an ischaemic heart. Furthermore, they illustrate the fundamental significance of the product of the union between basic and clinical science.

The rapid development of knowledge in the "Lipids-Heart-Heart Disease" triad prompted us to organize the International Symposium on Lipid Metabolism in the Normoxic and Ischaemic Heart, held in Rotterdam (The Netherlands) on September 22 and 23, 1986. This meeting was a coproject of the Department of Biochemistry I (Medical Faculty, Erasmus University Rotterdam) and the Department of Physiology (Medical Faculty, University of Limburg) and was held under the auspices of the Gerrit Jan Mulder Foundation (named after a famous Rotterdam physician and chemist who lived from 1802—1880) whom we thank for their co-operation.

The present volume is a compendium of invited papers and contributions selected from the posters presented during the symposium. It covers the state of the art and presents original experimental data on the following areas of myocardial lipid metabolism: (i) Lipids as fuel for the heart, (ii) Structural lipids of the heart, (iii) Ischaemia-induced alterations in myocardial lipid metabolism and (iv) Toxicity of lipid intermediates in the heart. In addition, it includes contributions on methodological aspects of lipid research, pharmacological tools and therapeutics, dietary fatty acids and heart function and on lipid peroxidation.

The editors of Basic Research in Cardiology and Dr. Dietrich Steinkopff Verlag are gratefully acknowledged for publishing the symposium proceedings, thereby enabling a more general availability for the presented material. Sigma Tau (Rome, Italy) is thanked for sponsoring this publication.

Finally, it is our sincere hope that the present report will stimulate further basic research in myocardial lipidology in an attempt to find answers to the many unanswered questions still left in this fascinating area and to bring us closer to a better understanding of myocardial metabolism and function, and the treatment of ischaemic heart disease.

H. Stam and G.J. van der Vusse.

Contents

I. Lipids as fuel for the heart

Substrates for energy metabolism in the heart: the role of the interstitial compartment

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Summary

Evidence is presented that, as in cardiomyocytes, vascular endothelial cells use fatty acids, in addition to glucose, as a respiratory fuel. Attention is focused on the cardiac interstitium, lined by vascular cells and cardiomyocytes, which may be enriched with metabolic products from these cells. Also, certain proteins are present in the interstitial fluid (Q_i) such as plasma proteins and fatty acid binding protein (FABP). However, the concentration of FABP is so low in Q_i that albumin is more important to shuttle long chain fatty acids in the interstitial fluid between cardiomyocytes and the vascular compartment.

Under hypoxic conditions (hypo)xanthine, lactate and fatty acids may be expected to accumulate in the interstitium, as well as proteins from adjacent cells, such as xanthine oxidase from endothelial cells. This enzyme, acting upon the elevated level of (hypo)xanthine, giving rise to $O_2^{-}\cdot$, may be involved in the damage of the ischaemic heart. The significance of the interstitium in ischaemia and in fibrosis following long standing cardiac lipidosis is briefly discussed, as well as the possible mechanisms involved in fatty acid transport in the heart.

Key words: Myocardial substrates, metabolic products, interstitium, fatty acid binding protein, lipoprotein lipase, ischaemia, fibrosis, carnitine.

Introduction

The heart is able to use a number of different substrates for aerobic energy production: glucose, fatty acids, ketone bodies and lactate (5, 16, 17, 20). The plasma level of the substrates largely determines the rate of entry of these substrates into the cardiomyocytes. The level of glucose varies only in a narrow range under physiological conditions. However, the rate of glucose entry is highly dependent upon the hormonal balance (the levels of insulin on one hand and of glucagon and catecholamines on the other) which, in a more remote sense, also determines the availability of other substrates. As for glucose transport, it has become most likely that insulin increases the number of glucose tansporters available for passage through the sarcolemma (9). There are also endogenous fuels stored in the myocardium such as glycogen and triglyceride. Glycogen is rapidly mobilized during ischaemia and triglyceride is continuously turning over during aerobic metabolism. Triglyceride increases during hypoxic perfusion.

Fatty acids are made available to the myocytes by the lipoproteinlipase reaction and by fatty acids bound to albumin in the blood. Fatty acid binding protein (FABP), present in all cardiac cell types and perhaps also in the interstitium (6), may be involved in fatty acid transport as well. The interstitial space will receive particular attention in the present communication as it is a zone of contact between the vascular space and myocytes.

Methods and materials

Hearts from recently-fed male Wistar rats of 250—300 g were used for perfusion according to the Langendorff technique. The perfusion buffer used was a modified Tyrode solution containing 128 mM NaCl, 4.7 mM KCL, 1.3 mM $CaCl_2$, 20.2 mM $NaHCO_3$, 0.4 mM Na_2PO_4, 1.0 mM $MgCl_2$ and 11 mM glucose. The medium was continuously gassed with 95% O_2 and 5% CO_2 (pH 7.4 at 37°C). The hearts were not stimulated electrically and the perfusion pressure was 85 cmH_2O. When indicated, coronary effluent (Q_{rv}) was separated from the interstitial fluid (Q_i) by the technique of De Deckere and Ten Hoor (3). Q_{rv} amounted to 10.5 ± 0.5 ml (n = 15) per min and Q_i (which drips from the apex) to 0.10 ± 0.01 ml per min (n = 15). The technique took about 18 min of preparation time. Q_i and Q_{rv} collection then took place. When indicated, the perfusion medium was enriched with Intralipid, containing 0.5 mM tri [9, 10(n)-^{3}H] oleoylglycerol (Amersham International plc, Amersham, U. K.), prior to Q_i and Q_{rv} collection. Intralipid (20%) was from Kabi-Vitrum (Stockholm, Sweden). After a 10-fold dilution with phosphate-buffered saline, it was added to the dry residue of tri [9, 10(n)-^{3}H]-oleoylglycerol in 2 ml portions in plastic vials and sonicated at 20 kHz for 30 s. Then the radioactive emulsion was incubated with pure human apolipoprotein C_{II} (a gift from Dr. P. H. E. Groot) for 1 h at 37°C to stabilize these artificial chylomicrons prior to dilution in standard perfusion medium. The final concentration of apolipoprotein in the perfusion medium was 0.035 mM and, when indicated, fatty acid free bovine serum albumin was 0.15 mM. The perfusate samples obtained were extracted with chloroform/methanol (2). The organic phase was evaporated to dryness and the residue chromatographed on silicic acid plates, developed with heptane/diethylether/acetic acid (60:40:1 by vol.). Fatty acids (Rf 0.4) (Rf = Retardation factor) and triglycerides (Rf 0.7) were scraped from the plates and counted in Instagel (Packard, Illinois, U. S. A.). DNA was determined by the method of Kapuscinski and Shoczylas (14). Electrophoresis was conducted on 12% polyacrylamide slab gels, containing 0.1% (w/v) sodiumdodecyl sulphate. This and the subsequent immunoblotting were carried out exactly as described (19). Rabbit antiserum against purified rat heart FABP (7) was kindly donated by Prof. Dr. J. H. Veerkamp (University of Nijmegen, The Netherlands).

Results and discussion

Energy metabolism of vascular endothelium

Fatty acids have a predominant role in energy metabolism of cardiomyocytes. This has been known for a considerable time (16, 17, 20). Their role in the energy supply of other cell types in the heart is not known. Vascular endothelium has been shown to contain coupled mitochondria and yet perform a high rate of aerobic glycolysis (4). An indication that fatty acids may be oxidized in endothelial cells may be inferred from the observation of Van Hinsbergh et al. (25) that cultured cells occasionally contain lipid droplets that may disappear after carnitine addition. It can be seen from Table 1 that a confluent culture of human arterial endothelial cells is able to oxidize added oleate in a cyanide-sensitive manner, and that in the presence of cyanide the cells accumulate triglyceride. The rate of fatty acid oxidation is considerable. Its calculated equivalency to ATP synthesis (assuming that the complete oxidation of 1 mol oleate can produce a net synthesis of 144 mol ATP from ADP and P_i) is much higher than that from the rate of aerobic glycolysis (cf. 4). A sufficient fatty acid supply might also be provided by lipoprotein lipase reaction, which is known to take place on the surface of vascular endothelial cells of the myocardium.

The presence of lipid carrier proteins and lipoprotein lipase in myocardial interstitium

Studies of cardiac cells showed that fatty acid uptake is a saturable phenomenon (18, 21). This also applies to liver and other cell types (cf. 1, 24). Immunohistochemistry of the heart studies by Fournier and Rahim (6) showed that FABP is present in all cells types of the heart as well as in the interstitial space. It could fulfil a role in intracellular transport as well as in the intercellular transport of fatty acids.

We have been able to detect FABP in the interstitial fluid of rat heart perfused by a modified Langendorff technique in which interstitial fluid (Q_i) can be separated from coronary effluent

Table 1. Fatty acid metabolism in cultured human arterial endothelial cells

Addition	*n*	Fatty acid oxidized (nmol/mg DNA/h)	Fatty acid incorporated into TG
none	3	53.6 ± 19.5	–
L-carnitinie (52 μM)	3	109.1 ± 2.6	–
+ 1 mM KCN	3	16.5 ± 4.1	107.6 + 9.6

Incubations were carried out at 37°C in an atmosphere of 95% O_2 + 5% CO_2 in 2 ml M/199 culture medium containing 20% (v/v) human serum, 5.5 mM glucose and 0.4 mM [^{3}H]-oleate. The incubations were terminated after 10—20 h by removal of the medium. This was treated with chloroform/methanol [2]. Duplicate samples were removed from the aqueous phase, one of which was counted directly and one after evaporation to dryness to determine ^3H_2O formation. When triglyceride formation was determined, the cells collected after brief sonication in 2 ml 0.5% (w/v) bovine serum albumin and also treated with chloroform/methanol [2]. The organic phase was evaporated to dryness and chromatographed for the isolation of triglycerides as described under Methods.

(Q_{rv}). After polyacrylamide gel electrophoresis of Q_i, followed by immunoblotting, a band of FABP (M_r : 15 kD) can be identified (Fig. 1). The concentration of FABP secreted or lost by leakage from cardiac cells into Q_i slowly decreases with time (Fig. 2). This could be due to a decreased rate of secretion (or leakage) into the interstitium, or to a dilution of interstitial fluid with vascular perfusate, due to a gradual loss of the endothelial barrier. The gradual decrease of Q_i constituents with time could also be demonstrated for 2 other proteins: one synthesized by

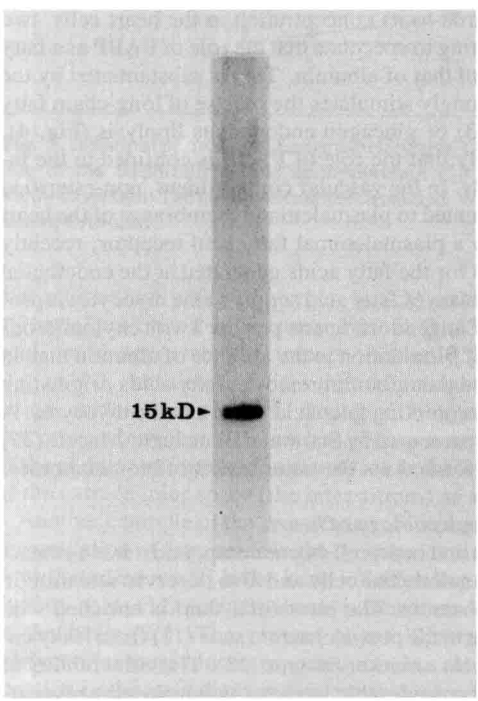

Fig. 1. Immunoblotting of interstitial fluid with anti-FABP. The molecular weight was determined with the aid of standards.

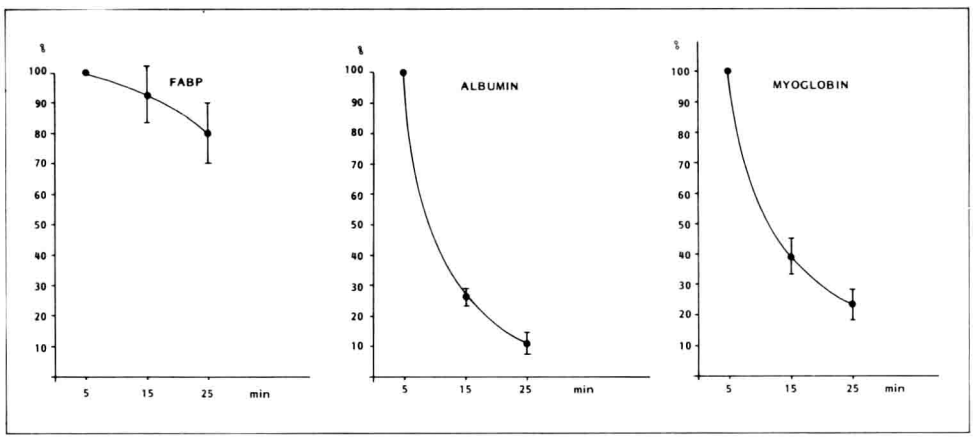

Fig. 2. Fatty acid binding protein, albumin and myoglobin in interstitial fluid. In each experiment (n = 3—5) three 10 min fractions of Q_i were collected. The amount of FABP in Q_i was quantified by counting the ^{125}I-protein A in Dimilume after immunoblotting of the samples by dissolving the FABP bands of the blot in Soluene (Packard) (Fig. 1.) The average of the first fractions was set at 100% and corresponds to approximately 0.03 mg/ml. Albumin was determined by radial immunodiffusion and myoglobin by its peroxidative activity, as employed for haemoglobin. The 100% values for albumin and myoglobin were 1.0 ± 0.1 mg/ml and 7.0 ± 0.2 μg/ml respectively.

cardiomyocytes (myoglobin) and one synthesized by the liver (albumin), as is also depicted in Fig. 2. The concentration of FABP in Q_i, as calculated by extrapolation of the curve in Fig. 2, is about 0.03 mg/ml, which is very low compared to its concentration in the heart cells, two orders of magnitude higher. Therefore it is tempting to speculate that the role of FABP as a fatty acid shuttle in Q_i must be negligible compared to that of albumin. This is substantiated by the finding that 0.15 mM bovine serum albumin strongly stimulates the release of long-chain fatty acids into Q_i during Intralipid perfusion (Fig. 3) or glucagon endogenous lipolysis (Fig. 4). From the results so far discussed, it seems likely that the role of FABP is confined to the intracellular compartment of the various heart cells. In the vascular compartment, non-esterified fatty acids are bound to albumin and will be presented to plasmalemmal membranes of the heart as such. Perhaps a subsequent role is played by a plasmalemmal fatty acid receptor, recently demonstrated by Stremmel (24), in the liver. As for the fatty acids generated at the endothelial surface of the cardiac capillaries, another mechanism of fatty acid supply to the myocytes is probably involved. Earlier (10) we have shown that Langendorff hearts perfused with chylomicrons labelled in the triglycerides, have a higher rate of β-oxidation in the absence of albumin than in its presence. From this experiment we concluded that albumin removes fatty acids originating from the lipoprotein lipase reaction, rather than promoting fatty acid entry into the myocytes. A possible mechanism that can enable this has been presented by Scow and Blanchette-Mackie (22) who suggest a lateral transport of the fatty acid formed via the outer leaflet of biomembranes.

The significance of the interstitium for the pathophysiology of heart

The interstitium is enriched with plasma-borne and heart cell-borne compounds. It is a contact zone of cardiomyocytes and capillary vascular endothelial cells and also deserves attention in pathophysiological studies. One example is ischaemia. The interstitial fluid is enriched with (hypo)xanthine (3) and contains xanthine oxidase in the post-ischaemic state (11) from leaky endothelial cells in which xanthine oxidase constitutes a marker enzyme (13). The vulnerability of endothelial cells in ischaemia (11) is therefore of considerable interest, as is makes the myocar-

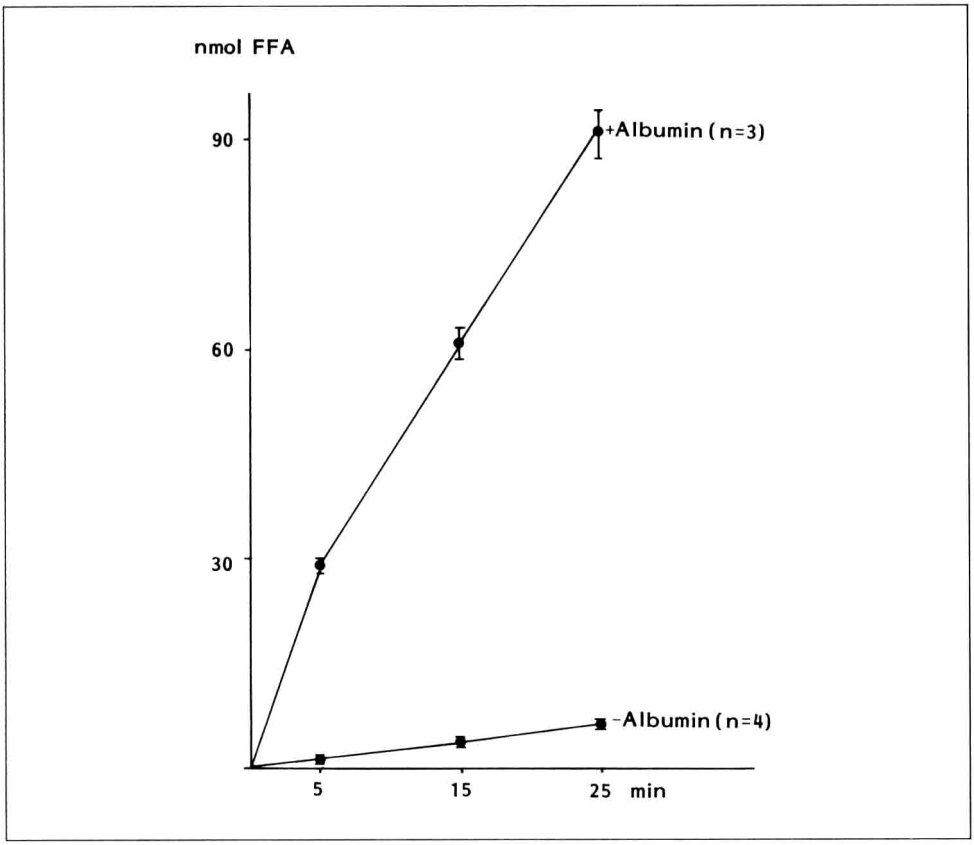

Fig. 3. Intralipid perfusion of Langendorff hearts. Q_i was collected in three 10 min fractions. For preparation of the ^{3}H-Intralipid (and apolipoprotein C_{II}) containing medium and the methods followed see Methods section. The media were not recirculated. When indicated albumin was present in a final concentration of 0.15 mM.

dial interstitium a site of oxygen radical production as, moreover, the interstitium contains myocytal products in high concentrations (3, 23), such as (hypo)xanthine and lactic acid. Lactic acid could aggrevate the impact, not only as the lower pH promotes the conversion of $O_2^{-}\cdot$ to $HO_2\cdot$ but also mobilizes Fe^{2+} from transferrin, allowing the formation of $OH^{\cdot}$ (8). In addition, acidosis can cause the damage of biomembranes. Hence it is conceivable that measures that prevent lactic acidosis, such as glycogen depletion, are protective (15). The protective effect of the addition of superoxide dismutase to the heart during low flow ischaemia (11) is indicative of the extracellular space (the interstitium) as an important locus of free radical formation.

Another example of the importance of interstitial products in cardiac pathology is the chronic accumulation of fatty acids in the lymphatics during lipolysis in hearts suffering from steatosis. A well-studied model is the heart after feeding of erucic acid-rich diets. The chronic irritation by fatty acids (12) probably leads to fibrotic reactions, confinded to the area of the lymphatics in the heart (26). The present paper illustrates that not only fatty acids mobilized from triglycerides stored in the myocardium, but also fatty acids derived from the lipoprotein lipase reaction in the vascular compartment are present in the interstitium.

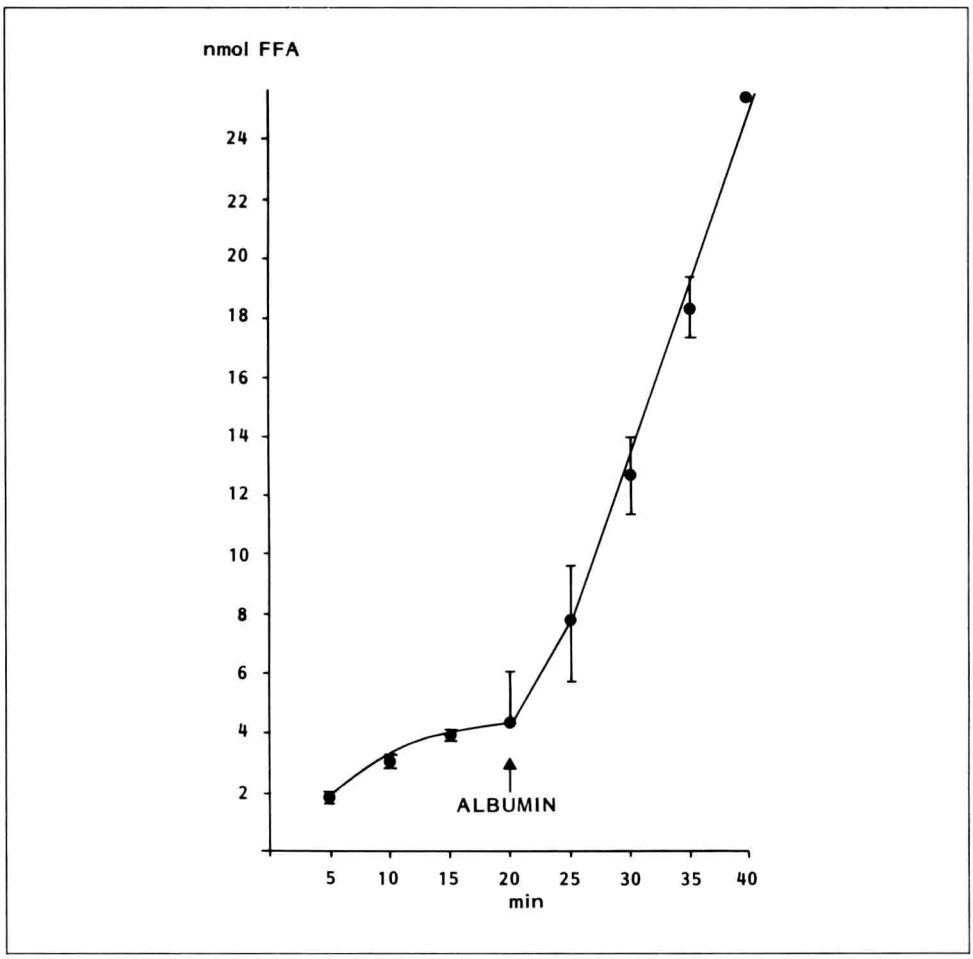

Fig. 4. Endogenous lipolysis of rat hearts during substrate-free perfusion of Langendorff hearts in the presence of glucagon. Q_i was collected continuously after glucagon (2×10^{-7} M) addition. At t = 20 min fatty acid-free albumin (0.15 mM) was introduced. See also Methods section.

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