INFLUENCES OF DIETARY LINOLEIC ACID ON CORONARY FLOW, LEFT VENTRICULAR WORK, AND PROSTAGLANDIN SYNTHESIS IN THE ISOLATED RAT HEART

PROEFSCHRIFT

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Promotoren:	Prof. Dr. W.C. Hülsmann
	Prof. Dr. W.G. Zijlstra (Groningen)
Coreferenten:	Prof. Dr. I.L. Bonta
	Prof. Dr. F. ten Hoor (Maastricht)

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"Failure is not failure
nor waste wasted,
if it sweeps away illusion
and lights the road to a plan".

H.G. Wells, 1911.

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CONTENTS

LIST OF ABBREVIATIONS AND SYMBOLS

ADP	Adenosine-5'-diphosphate
ASA	Acetylsalicylic acid
cAMP	Cyclic adenosine-3',5'-monophosphate
CoA	Coenzyme A
ECG	Electro cardiogram
EDTA	Ethylenediaminetetra-acetic acid
EFA	Essential fatty acid
en%	Energy per cent: per cent of the amount of digestible energy
f_p	Pacing or stimulation frequency of the heart
f_s	Spontaneous heart frequency
FA	Fatty acids
FFA	Free fatty acids
GLC	Gas liquid chromatography
HCO	Hydrogenated coconut oil
HSBO	Hydrogenated soyabean oil
KH	Krebs-Henseleit
L	Lard
LSO	Linseed oil
m_b	Body mass
m_h	Dry heart mass
OO	Olive oil
$\bar{P}_{ao}$	Mean aortic pressure
P_{aoh}	Hydrostatic aortic pressure
P_{aop}	Peak aortic pressure
P_{lvf}	Left ventricular filling pressure (~ mean left atrial pressure)
P_{lvs}	Left ventricular systolic pressure
P_{O_2}	Oxygen tension
P_p	Perfusion pressure
PG	Prostaglandin
PGI_2	Prostacyclin
PO	Palm oil

$\dot{Q}_{cor}$	Coronary flow rate
$\dot{Q}_{lv}$	Left ventricular output
Q_{O_2}	Oxygen consumption
SBO	Soyabean oil
SD	Standard deviation
SEM	Standard error of the mean
SSO	Sunflowerseed oil
t	Time, usually the time from the start of perfusion
T_3	3, 5, 3'-Triiodothyronine
T_4	3, 5, 3', 5'-Tetraiodothyronine (thyroxine)
TG	Triacylglycerol
TLC	Thin layer chromatography
TLG	Trilinoleoylglycerol
TPG	Tripalmitoylglycerol
TX	Thromboxane
$\dot{V}_{O_2}^m$	Myocardial oxygen consumption
W_{lv}	External left ventricular work
$W_{lv}(max)$	Maximal value of W_{lv} reached by increasing P_{lvf}

1. INTRODUCTION AND LITERATURE SURVEY

1.1 INTRODUCTION TO THE THESIS

Because of the frequent occurrence of ischemic heart disease, interest in the consequences of dietary fat intake for the heart has increased strongly, and many studies have shown the importance of dietary fat for the heart. Most studies deal with the role of dietary fat in coronary atherosclerosis and the incidence of ischemic or coronary heart disease due to the obstruction of the coronary arteries by atherosclerotic plaques. Several studies have shown that dietary fat can also affect the myocardial muscle cell directly. The best known and most extensive studies in this field are those of essential fatty acid deficiency and dietary rapeseed oil containing a high amount of erucic acid. Only few studies on the effects of other dietary fats on the heart, in which atherosclerosis is not involved, have appeared. However, the interest in direct effects of dietary fat on the heart is growing.

The final objective of the present study is to gain information about possible favourable and unfavourable properties of dietary fats used for human consumption. The particular objectives of the study were to investigate influences of fats fed to rats for a short period of time on coronary flow rate and external left ventricular work in their isolated, perfused hearts. As the effects found could be ascribed to dietary linoleic acid, which is the ultimate precursor of the prostaglandins of the 1- and 2-series, it was investigated whether prostaglandin synthesis in the isolated heart was involved.

The build-up of the thesis is as follows. Chapter 1 gives a review of the effects of dietary fat on the heart (excluding atherosclerosis), as well as a review of the differences found in the heart in the oxidation rates and metabolism of various long-chain fatty acids. Chapter 2 contains methods and procedures and a discussion on the use of the isolated, working rat heart for physiological investigations. A discussion on expressing the heart function parameters used per unit of dry heart mass is given in chapter 3. The investigations into the effects of various dietary fats and of prostaglandins in the isolated heart are presented in chapters 4-6 and in the Addenda. The overall discussion on the influences of the dietary fats used on coronary flow rate and external left ventricular work, and on the role of prostaglandins is given in chapter 7.

1.2 EFFECTS OF DIETARY FAT ON THE HEART

1.2.1 Introduction

During the last 3 decades many studies have demonstrated the role of dietary fat in the etiology of ischemic heart disease. Ischemic heart disease can be regarded as an indirect effect of dietary fat, as it is usually caused by atherosclerosis of the coronary vessels. However, numerous data in the literature show that dietary fat can also directly affect the myocardial muscle cell resulting in changes in heart function. This survey deals mainly with such direct effects of dietary fat, studied in the isolated, perfused heart, though there is also an extensive literature on effects of other dietary components and various dietary regimens; e.g. influences of vitamin deficiency [5,14,33,43,45,61] and alcohol [47] on the heart have been found. Also a dietary regimen like excessive caloric restriction affects myocardial metabolism [19]. Many changes in enzyme activities have been observed. However, moderate caloric restriction is not necessarily injurious to the heart [55]. Many studies have been made of the effects of starvation on the heart. Starvation for a number of days increases the myocardial glycogen and triacylglycerol (TG) contents as well as the turnover and utilization of endogenous lipids [11,28,36,59].

1.2.2 Essential fatty acid deficiency

Essential fatty acid (EFA) deficiency has been found to cause changes in the ECG [4], enlargement of the myocardial mitochondria [52], a drop in the ADP/O ratio [6] and a decrease in total lipids in mitochondria [6]. The amount of phospholipids (PL) in mitochondria or in whole heart tissue is, however, not affected [6,52]. The amounts of linoleic acid (C18:2(n-6)) and arachidonic acid (C20:4(n-6)) in the PL decrease, being mainly replaced by oleic acid (C18:1(n-9)) and eicosatrienoic acid (C20:3(n-9)) [6,10]. Maybe, EFA deficiency increases the turnover of PL [8,52]. The literature on the effects of EFA deficiency on the heart up to 1970 has been reviewed by Del Rosario [10]. General reviews on EFA deficiency have been written by Holman [21] and Guarnieri and Johnson [15]. The use of EFA deficiency in studying roles of prostaglandins is evaluated by Parnham et al. [41]. For effects on myocardial contractile force see *1.2.4.3.*

1.2.3 Dietary fish oil

Like rapeseed oil (see *1.2.4.2*), fish oils or partially hydrogenated fish oils containing docosamonoenoic acids (C22:1) caused the myocardial lipid content to increase [56]. The increase was proportional to the dietary amount of C22:1. Owing to the very long-chain poly-unsaturated fatty acids occurring in marine oils, diets containing such oils can have serious impacts on the heart. By feeding diets containing cod-liver oil, linoleic and arachidonic acid are replaced by docosahexaenoic acid (C22:6(n-3)), as a result of which myocardial prostaglandin synthesis may be reduced due to a decrease in substrate or to inhibition of prostaglandin synthesis by the n-3 fatty acids. Hearts of rats

fed cod-liver oil were much more sensitive to depletion of high energy phosphate stores by isoproterenol than hearts of control rats which led to an increase in myocardial necrosis and mortality in the cod-liver oil group. It was suggested that the replacement of arachidonic acid by docosahexaenoic acid was responsible for this phenomenon [16-18]. For a review of the general aspects of feeding poly-unsaturated fats such as occurring in fish oils see reference 23.

1.2.4 Dietary fat and heart function

1.2.4.1 Introduction

In the study of the influences of dietary fat on the heart, two main types of investigation have been carried out:

- a high-fat diet has been compared with a low-fat diet;
- one type of fat has been compared with another.

The interpretation of the results of the first type of investigation can be difficult, as they may have been influenced by the specific properties of the fat or by a change in the energy balance as a result of replacing fat by carbohydrates. In several of the investigations reviewed [44,46,47,69], the interpretation of the results is still more complicated as the diets which were compared differed in more than one respect. Below, both types of investigations will be reviewed, with emphasis on the influence on heart performance.

1.2.4.2 Rapeseed oil

Of all the dietary fats investigated as to their effects on the heart, rapeseed oil containing a high amount of erucic acid (C22:1(n-9)) has been investigated the most extensively. Feeding this fat to rats increases the amount of TG in the heart [1,24], probably because of the inhibition of carnitine-acyl CoA transferase [62] and an increase in lipoprotein lipase activity [26]. The TG content in the heart is highest after 3 days of feeding a rapeseed oil-containing diet and then decreases again, but it remains higher than that in hearts of rats fed diets with a high content of sunflowerseed oil [24]. This decrease in TG content is most probably caused both by an induction of peroxisomal fatty acid oxidation [35] and an increase in the rate of TG hydrolysis [51]. Feeding rapeseed oil containing a large amount of erucic acid can have various impacts on the heart of experimental animals. Only the effects on coronary flow rate and myocardial contractile force will be discussed here. A comprehensive review on the effects of dietary rapeseed oil has been written by Vles [63].

In experiments performed by Ten Hoor *et al.* [58] groups of rats were fed diets containing 50 per cent of the digestible energy (50 en%) as rapeseed oil (containing 46% (w/w) erucic acid) or 50 en% sunflowerseed oil for 3 days. It was found that rapeseed oil, in contrast with sunflowerseed oil, lowered the maximum value of left ventricular stroke work in the heart-lung preparation, as well as the maximum developed tension and the rate of tension development in the papillary muscle. The authors ascribe the effects of rapeseed oil to a reduced rate of ATP synthesis, as observed in isolated heart mitochondria of rats fed rapeseed oil (46% erucic acid) [22]. On the other hand, the hearts of

the rapeseed oil-fed rats contained about 5,7% of tissue mass TG as against 0,8% in the hearts of the control group. This high increase in TG content has also been found by others [24,39,51]. Thus, it may be that feeding rapeseed oil increases the myocardial wall volume [64] and thus influences external left ventricular work in the heart-lung preparation.

1.2.4.3 Essential fatty acid deficiency

In experiments by Ten Hoor *et al.* [57,58] rats were made EFA deficient by feeding a diet free from linoleic acid for 6 months. In the papillary muscle preparation of these animals the maximum developed tension and the maximum rate of tension development were significantly lower than in the papillary muscle of control rats. Also other authors [8] found that by EFA deficiency myocardial contractile force and spontaneous heart frequency was decreased.

1.2.4.4 High-fat atherogenic diets

In the following studies, rats and rabbits were fed high-fat atherogenic diets and the effects on heart performance were investigated.

In a study by Reid *et al.* [46] two groups of rats were fed for about 4 weeks a commercially available low-fat diet (19% (w/w) protein, 61% carbohydrate and 4% fat) or a high-fat diet consisting of 60% of the commercial diet, 32% beef tallow, 6% olive oil and 2% cholesterol. In the Langendorff-perfused heart, uptake and oxidation of glucose were lowered by the high-fat diet. Mechanical heart performance was determined by a force transducer attached to the heart (preload 10 g). Neither with glucose nor with palmitate as external substrate were differences found in the mechanical heart performance of the two dietary groups.

In a study by Segel *et al.* [47] rats were fed a diet containing 7.4 en% fat (low-fat diet) or 20.4 en% fat (high-fat diet) for 34 weeks. In the low- and high-fat diets 7.4 en% fat was derived from a chocolate base. To the high-fat diet 9 en% olive oil and 4 en% corn oil were added. The values found for the left ventricular systolic pressure and ejection velocity in the isolated, working heart did not differ for the two groups. However, these parameters were measured at an end-diastolic pressure of about 0.38 kPa (2.85 mm Hg), which is probably too low to detect differences. During hypoxia, the maximum left ventricular systolic pressure and cardiac output were higher in the low-fat group.

Zbinden and Rageth [69] fed female rats 40% (w / w) butter + 5% cholesterol + 2% sodium cholate + 53% of a commercial diet (high-fat diet) for 1 and 2 weeks. The control group received the commercial low-fat diet. Left ventricular output and blood pressure were measured in the anesthetized (urethane) rat. The rats fed the high-fat diet had lost weight during the feeding period; the wet heart mass being about 75% of that of the control group. Feeding this diet lowered the cardiac output and the responsiveness to catecholamines.

Also, disturbances in the ECG were recorded. It was concluded that the changes had been caused by the dietary fat. However, any of the many differences between the two diets could have been the causative factor. Moreover, cardiac output was not corrected for differences in heart mass and the end-diastolic pressure was not measured.
Peterson *et al.* [44] fed to one group of rabbits pellets (control diet) and to another group an atherogenic diet composed of 89.5% (w/w) rabbit pellets, 5% lard, 5% peanut oil and 0.5% cholesterol. The diets were fed for 2 to 8 months. In the right papillary muscle preparation of the rabbits fed the atherogenic diet, a decrease in the contractile properties were observed at a contraction frequency of 12 min^{-1}. However, at a frequency of 24 min^{-1} the differences between the groups were smaller, and after addition of norepinephrine to the bath solution the differences were reduced to nil. The decrease in contractility occurred mainly during the first 2 months of feeding the atherogenic diet and was not correlated with the incidence of atherosclerosis. According to the authors, the decrease in contractility is due to a change in cellular membranes, induced by incorporation of cholesterol. This results in a decreased activity of membrane-bound enzymes and leads to a change in the calcium fluxes. However, feeding cholesterol has also a number of other effects. It was found to decrease the TG content in hearts of rabbits and guinea pigs and the amount of poly-unsaturated fatty acids in hearts of guinea pigs and rats [2,42,67]. Consequently, the observed decrease in contractility might be due to a decrease in endogenous substrate or to a change in the fatty acid composition of the PL.
A few additional remarks should be made on the diets used in the last 4 studies reviewed [44,46,47,69]. In these studies the fat content of the diets was increased simply by adding fats to the basal diets, leading to an increase in the energy content. As a result, the animals consumed less of the basal diet components, leading to lower protein, mineral and vitamin intake. This may have resulted in a deficiency of these components and thus have influenced the results. Therefore, the effects found cannot be ascribed solely to the large amount of fat in the diets. Moreover, in 3 out of the 4 studies cholesterol was fed. Yokoyama and Henry [68] found that in the isolated canine coronary artery, cholesterol in picomolar concentrations is an efficacious factor for vasoconstriction. It is possible that this property of cholesterol together with an increase in the amount of cholesterol in the heart are responsible for some of the effects observed. In any case, these 4 studies show that atherogenic diets containing large amounts of saturated fats and cholesterol, as a result of which the intake of essential dietary components may be insufficient, can affect the mechanical performance of the heart.

1.2.4.5 Type of dietary fat and substrate oxidation
The following 2 studies show that the type of fat fed may influence the oxidation of substrates in the heart as a result of which mechanical heart performance could be affected.

Mathias *et al.* [32] fed rats a low-fat diet (2 en% corn oil) and two high-fat diets containing 2 en% corn oil + 40 en% beef tallow and 42 en% corn oil, respectively. After several months of feeding, hearts were isolated and homogenized. Samples of the homogenates were separately incubated with ^{14}C-labelled palmitic, oleic or linoleic acid. The oxidation rate of the fatty acids was determined by measuring the amount of $^{14}CO_2$ developed. After feeding corn oil, the oxidation rates of the fatty acids were higher than after feeding beef tallow or the low-fat diet, although the differences were not statistically significant.
In a study by Hsu and Kummerov [27] 4 groups of rats were fed for 1 to 6 weeks diets containing 22% corn oil, rapeseed oil or partially hydrogenated fat (49% C18:1 trans; 3% C18:2), or 20% hydrogenated fat + 2% corn oil. The oxidation rates of pyruvate, glutamate and the carnitine esters of oleic, erucic and elaidic acid were determined in the isolated heart mitochondria. For the mitochondria of the rats fed corn oil, the rates of oxygen consumption and ATP synthesis (with all substrates used) were significantly higher than for mitochondria of the rats of the other three dietary groups; between the three latter groups no statistically significant differences were found. It is not clear whether in this study the amount of fat fed is expressed in mass or energy per cent and whether the C18:2 in the hydrogenated fat was cis-cis linoleic. Moreover, ADP / O ratios higher than 3 were frequently obtained.

1.2.5 Dietary fat and fatty acid composition of myocardial phospholipids
From many investigations [6,10,12,17,18,25,39,54] it appears that the dietary fatty acid composition influences the fatty acid composition of the myocardial PL. As the activities of membrane-bound enzymes may be influenced by the fatty acid composition of the PL [13] and as prostaglandins are derived from arachidonic acid present in the PL of the membranes, it is important to know to what extent the fatty acid composition of the PL is influenced by the dietary fat used. There are considerable differences in the degree of incorporation of fatty acids from the various dietary fats into the myocardial PL. For instance, in feeding experiments with coconut oil, lauric acid and myristic acid were not incorporated into the myocardial PL [10]. Also, in red blood cells lauric acid is hardly incorporated into the PL, whereas linoleic acid is rapidly incorporated [34]. In the isolated, perfused rat heart linoleic acid is incorporated into the PL to a greater extent than other long-chain fatty acids [52,60]. However, linoleic acid and also arachidonic acid are displaced in their turn by docosahexaenoic acids (C22:6) present in dietary cod-liver oil [17,18]. In the rat heart, a positive linear relationship was found between the amounts of certain fatty acids (e.g. C22:6, C18:2, C18:1) in the TG and those in the PL; in the PL, the more unsaturated fatty acids seemed to be able to displace the less unsaturated ones [17].
Del Rosario [10] made an extensive study of the effect of dietary fat on the fatty acid composition of myocardial PL. Sprague Dawley weanling rats were fed 20 en% fat for 6 weeks. Four types of fat, containing 4 different levels of

linoleic acid, were fed: 20 en% hydrogenated coconut oil (0 en% C18:2(n-6)), 10 en% hydrogenated coconut oil + 10 en% coconut oil (0.5 en% C18:2 (n-6)), 13.4 en% coconut oil + 6,6 en% corn oil (4.0 en% C18:2(n-6)) and 20 en% corn oil (12 en% C18:2(n-6)). The amount of dietary linoleic acid was found to have no effect on the relative amounts of the various PL classes in the heart. The PL classes are shown in Table 1.1. With increasing amount of linoleic acid in the diet, the amounts of linoleic and arachidonic acid (mainly in the β -position) increased at the cost of oleic acid and eicosatrienoic acid (C20:3(n-9)) (Tables 1.2 and 1.3). However, the amount of arachidonic acid in the PL classes, except maybe in phosphatidylcholine, did not increase further at more than 4 en% dietary linoleic acid; at 4 and 12 en% linoleic acid the total amount of arachidonic acid in the PL was about 23%. Increasing the amount of dietary linoleic acid hardly affected the unsaturated FA / saturated FA ratio in total PL, with the exception of an increase in phosphatidylethanolamine. In the PL of the liver, the effect of the amount of dietary linoleic acid was different from that in the heart PL, not only as a result of differences in the relative amounts of the PL classes between liver and heart (Table 1.1), but also because of differences in the degrees of incorporation of the various fatty acids.

TABLE 1.1 Phospholipid classes (%; w/w) in rat heart and liver.

Class	Heart	Liver
Phosphatidylcholine (PC)	36.6	51.9
Phosphatidylethanolamine (PE)	32.5	25.9
Cardiolipin (C)	16.6	5.2
Phosphatidylinositol (PI)	4.5	8.2
Sphingomyelin (S)	4.0	4.0
Phosphatidylserine (PS)	2.7	2.6
Lysophosphatidylcholine (LPC)	2.7	2.6

Percentages were calculated from the results obtained by Del Rosario [10].

TABLE 1.2 Effect of the amount of dietary linoleic acid on the amount of linoleic acid (%; w/w) in the various PL classes of the rat heart.

Class	Dietary linoleic acid (en%)			
	0.0	0.5	4.0	12.0
PC	5.8	9.3	17.3	17.4
PE	3.6	5.1	11.3	15.1
C	59.8	63.2	78.9	93.3
PI	1.2	2.2	10.3	13.3
S	—	1.9	1.0	1.3
PS	7.4	16.8	39.6	46.5
LPC	5.9	6.4	16.8	12.1

For abbreviations see Table 1.1. Values found by Del Rosario [10]. The experimental diets were fed for 6 weeks.

TABLE 1.3 Effect of the amount of dietary linoleic acid on the amount of arachidonic acid (%; w/w) in the various PL classes of the rat heart.

Class	Dietary linoleic acid (en%)			
	0.0	0.5	4.0	12.0
PC	6.0	10.4	25.9	30.2
PE	20.7	27.4	31.6	29.6
C	—	—	—	—
PI	9.6	12.9	29.2	22.3
S	11.3	11.1	12.2	10.1
PS	6.5	10.4	13.5	12.7
LPC	—	6.4	17.8	16.6

For legends see Table 1.2.

1.2.6 Possible effects of dietary fat on myocardial infarction

The results obtained by Gudbjarnason and coworkers with fish oil-containing diets [17,18] show that the type of dietary fat influences the heart *in vivo* under certain conditions. Such a condition might also be myocardial infarction in man. During myocardial infarction the serum free fatty acid (FFA) level strongly rises, and it has been suggested that this could be the cause of the serious arrhythmias and disorders of conduction observed during myocardial infarction [29]. This was confirmed in experimental myocardial infarction studies in dogs [30]. However, other authors could not find any relation between the levels of serum FFA and the incidence of arrhythmia [37,38].

It might be that not only the level of serum FFA, but also the type of FFA released from adipose tissue during myocardial infarction or intravenously infused (Intrapalid®) influences the degree of infarction, including the occurrence of arrhythmia. In isolated rat hearts perfused with palmitic or oleic acid and albumin, high FFA / albumin molar ratios (2.5 or higher) caused serious arrhythmia [66]. Although no differences were found in the incidence of arrhythmia caused by the two fatty acids, the contractile force of the hearts perfused with palmitic acid decreased much quicker than that of the hearts perfused with oleic acid, which shows that high concentrations of palmitic acid are more harmful to the heart than high concentrations of oleic acid. In isolated perfused rabbit hearts, perfusion with stearic acid (FA / albumin molar ratio = 1) depressed the heart, whereas perfusion with oleic acid did not [48]. In papillary muscle preparations, linoleic or octanoic acid (+ albumin) decreased the contractile force during hypoxia; linoleic acid to a lesser extent than octanoic acid [20]. During hypoxia or ischemia, shortening of the action potential duration may occur. Shortening of action potential duration increases the susceptibility to cardiac arrhythmia and is potentiated by FFA. In the isolated guinea pig heart, Cowan en Vaughan Williams [9] found that linoleic acid (0.6 mmol l^{-1} + 0.14 mmol albumin per l) decreased the action potential

duration to about the same extent as palmitic acid, but to a lesser extent than octanoic acid (0.5 mmol l^{-1}). During ischemia the concentration of acyl-CoA esters increases rapidly [50,65]. Long-chain acyl-CoA esters inhibit the transport of ATP across the mitochondrial membrane. The inhibition by myristoyl- and oleoyl-CoA was found to be stronger than that by stearoyl- and palmitoyl-CoA; linoleoyl-CoA showed the weakest inhibiting effect [7,31,40,49]. When in the isolated, perfused frog heart the contractile force was decreased by an excess of Mg^{2+}, perfusion with fatty acids resulted in an increase in the contractile force, linoleic acid causing a much greater increase than oleic, lauric or octanoic acid [3].

All these data suggest that unsaturated fatty acids are less harmful to the heart than saturated fatty acids. Therefore, as the fatty acid composition of adipose tissue reflects that of the diet, it is possible that the fatty acids mobilized from adipose tissue during infarction are less harmful to the heart after prolonged diets rich in linoleic acid than after diets rich in saturated fatty acids.

1.2.7 Conclusion

This survey shows that several investigations have already been made on the effects of dietary fat on the functioning of the heart. The effects of EFA deficiency and rapeseed oil have been investigated the most extensively and the results show that the mechanical performance of the heart is depressed by EFA deficiency and by feeding rapeseed oil containing a large amount of erucic acid. However, investigations in which various dietary fats or different amounts of fat were compared with each other are scarce. The studies reviewed show that feeding fish oil, cholesterol or high-fat atherogenic diets affect the heart adversely, but exact data on their influence on the mechanical performance of the heart and on the mechanisms involved are not available. Possible mechanisms are e.g. an influence of dietary fat on substrate oxidation, differences in inhibitory actions exerted by the various long-chain acyl-CoA esters or changes in the fatty acid composition of the myocardial phospholipids.

During myocardial infarction the plasma free fatty acid level strongly rises. As the effects of the various long-chain fatty acids on the heart differ, the type of dietary fat may influence the course of the disease as a result of its influence on the type of the free fatty acids in the plasma.

1.2.8 References

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1.3 RELATIVE OXIDATION RATES OF LONG-CHAIN FATTY ACIDS IN THE RAT HEART

1.3.1 Introduction

It is well documented that dietary fat influences the fatty acid composition of the triacylglycerols (TG) in the heart [1,19,26,36,55,60,61]. The TG fatty acids are the main endogenous energy source of the heart and numerous studies have shown that the isolated rat heart, perfused with [7,30,44] or without [6,7,34,44] exogenous substrate, uses the TG fatty acids efficiently. The TG content is normally about 13 μmol per g of dry heart mass [6,10,34,43], but e.g. fasting during a few days [10,21] and feeding high amounts of docosamonoenoic acids, e.g. erucic acid [16], increase the TG content of the heart. Another endogenous substrate, but of lesser importance than the TG fatty acids, is glycogen. Normally, the glycogen content of the rat heart is 45 to 125 μmol glucose equivalentes per g of dry heart mass [6,29,35,43,44]. Glycerol, obtained from the hydrolysis of the TG, and the phospholipids are not or hardly used as substrate for the energy production in the heart [21,34].

As already mentioned, the endogenous TG fatty acids are very important for the ATP generation in the isolated rat heart. Even with exogenous glucose

present, the endogenous TG fatty acids can account for more than 50% of the oxygen consumption [30]. The use of the TG fatty acids is stimulated by catecholamines [8] or by an increase in external left ventricular work [6]. It might be possible that, particularly under conditions of a high rate of use of the TG fatty acids as in the case of high cardiac loads, the ATP generation needed for cardiac work is affected by the various kinds of fatty acids incorporated into the TG. Therefore, data on the relative oxidation rates of different fatty acids released from the TG would be of interest, but no such data are available, although various methods have been described to enrich the TG with radioactively labelled fatty acids [8,15,44,51,52]. However, results from perfusion experiments with various fatty acids in which uptake, esterification and oxidation of fatty acids were determined are available (see below).

As background information for the interpretation of the results from the investigations on the effects of dietary fats on the heart, a review of the uptake and oxidation of various fatty acids by the isolated rat heart is presented. Excellent reviews about the general aspects of the lipid and carbohydrate metabolism in the heart have been published by Neely *et al.* [30,31], Crass III [9] and Williamson [58]. The present review is limited to the literature on the relative oxidation rates of the various long-chain fatty acids and possible explanations of the differences in the oxidation rates observed.

1.3.2 Considerations on perfusion with fatty acids

Most of the studies on the relative oxidation rates of various long-chain fatty acids are difficult to interpret, because of differences between the long-chain fatty acids in solubility, binding to albumin, transport across the capillary wall or the myocardial cell wall [42], binding to the fatty acid binding protein of the cytoplasm [14, 28, 32] and rate of incorporation into the various lipids of the heart. Moreover, the data in the literature concerning fatty acid uptake and oxidation rates are not always comparable, as fatty acid perfusions with or without glucose in the perfusion fluid and perfusions with one fatty acid or with a mixture of fatty acids have been performed. In addition, the purity of the fatty acids may have differed, and the dietary fat fed before heart perfusion was generally not taken into account. The latter aspect is of importance, because feeding different fats will result in different compositions of the TG fatty acids. This probably will influence the results since in perfusion experiments with fatty acids the exogenously supplied fatty acids will mix with the endogenous fatty acids.

1.3.3 Perfusion of the isolated heart with various fatty acids

In the following studies the fatty acids in the perfusion fluid were bound to albumin, and the perfusion fluid was recirculated through the heart for a cer-

tain period of time. In most cases 1-^{14}C labelled fatty acids were used and the incorporation of label into the lipids was determined. The uptake was calculated from the disappearance of the fatty acids from the perfusion fluid, and the oxidation was calculated from the $^{14}CO_2$ production.

Evans *et al.* [11] and Evans [12,13] found that uptake and oxidation of long-chain saturated fatty acids decreased, and incorporation into tissue lipids increased with increasing chain length. The up-take of the monoenoic fatty acids palmitoleic and oleic acid was higher than that of the corresponding saturated fatty acids. The uptake of linoleic acid was less than that of oleic acid, but higher than that of stearic acid. The rate of oxidation of oleic acid exceeded that of all the other fatty acids. The fatty acids which were least well oxidized tended to be more readily incorporated into the tissue lipids.

Stein and Stein [50] found that the uptake rates of palmitic, stearic, oleic and linoleic acid were similar. About 70% of the fatty acids incorporated into total heart lipid was found in the triacylglycerols and about 30% in the phospholipids. Linoleic and stearic acid were incorporated into the phospholipids to a larger extent than palmitic and oleic acid. About 70% of the fatty acids incorporated into the phospholipids was found in phosphatidylcholine.

Willebrands and coworkers found for the uptake of fatty acids from the perfusion fluid the following ranking: oleate > linoleate = palmitate > stearate [56] and linoleate > elaidate [57]. Elaidic acid was found to be converted into trans-5-tetradecenoic acid, which was not or sluggishly metabolized, and released by the heart.

Rodis and Vahouny [41] found the uptake of palmitic acid to be greater than that of linolenic acid. Moreover, in percentage of uptake more palmitic than linolenic acid was incorporated into the triacylglycerols, and more linolenic than palmitic acid into the phospholipids. About 0.6% of the palmitic and 14% of the linolenic acid taken up was found in the free fatty acid pool. In percentage of uptake, linolenic acid was oxidized faster than palmitic acid.

Vasdev and Kako [54] found the following ranking for the fatty acid uptake: linoleic acid > linolenic acid > palmitic acid = oleic acid > erucic acid. For the fatty acid incorporation into total lipid (in % of the uptake) they found: erucic acid > palmitic acid = oleic acid > linoleic acid > linolenic acid; for the oxidation (in % of the uptake) they found the reversed ranking order: linolenic acid > linoleic acid > palmitic acid = oleic acid > erucic acid. The incorporation of linoleic acid or linolenic acid into the phospholipids was about 20% of the amount incorporated into the total lipid fraction; for the other three fatty acids this was about 6%. The fatty acids incorporated into the phospholipids were preferentially incorporated into phosphaditylcholine, except oleic acid which was incorporated preferentially into phosphatidylserine. Especially linoleic acid was incorporated into phosphatidylcholine to a high percentage.

About 11% of the linolenic acid and 21% of the erucic acid was found in the free fatty acid pool. For the other three fatty acids acids this was about 5%.
Gloster *et al.* [15] studied the time course of the incorporation of palmitic and oleic acid into different subcellar fractions. As soon as 5 s after the start of the perfusion about 20% of both palmitic and oleic acid taken up in the cells was found to have been esterified, and after 1 min these percentages were about 37 and 49 respectively. Moreover, palmitic acid was taken up to a larger extent than oleic acid. Therefore, the esterification of palmitic acid proceeded more slowly. Stein and Stein [51] performed similar experiments, but only with oleic acid. They found a higher esterification rate for oleic acid than Gloster *et al.* [15].
In Langendorff hearts perfused with palmitate or octanoate, Oram *et al.* [37] found that, when cardiac activity was increased by increasing the perfusion pressure, octanoate oxidation, but not palmitate oxidation, was fast enough to maintain high levels of acetyl-CoA. They concluded that the translocation of acyl units across the inner mitochondrial membrane is rate-limiting in the overall oxidation of fatty acids.
This review shows that the literature data on the ranking order of the uptake and oxidation rates of various fatty acids is rather inconclusive. From the data it appears that the uptake of fatty acids is not a measure of the oxidation rate. Only few studies have been performed [12,41,54] in which the oxidation rates of the different fatty acids were compared. From these data it follows that linoleic acid and linolenic acid are oxidized at a higher rate than the other fatty acids. Linoleic and linolenic acids are also incorporated into the phospholipids to a greater extent [41,50,54] than the other fatty acids, except perhaps stearic acid [50]. That the cellular free fatty acid concentrations are rather high when linolenic or erucic acid are used is remarkable [41,54].

1.3.4 Incubation studies with various fatty acids

A number of other methods have been used for studying the fatty acid metabolism of the rat heart. Vasdev and kako [54] used heart slices, incubated with radioactively labelled fatty acids for 30 min. The ranking order of the incorporation into total lipid and into the free fatty acid pool was: erucic > stearic > palmitic > oleic acid. The incorporation of erucic acid into total lipid was 5 times that of oleic acid. In heart homogenates [25] the ranking order of the relative rates of oxidation of uniformly- or 1-^{14}C labelled fatty acids was palmitic > linoleic acid > oleic acid. In isolated heart mitochondria Osmundson and Bremer [38] found that the oxidation rate of the carnitine esters of C18:1, C 20:1 and C22:1 decreased strongly with chain length. The oxidation rate of saturated fatty acid acylcarnitines was equal for chain lengths of 12, 14 and 16 C atoms and lower for chain lengths of 18 and 20 C atoms. Also in iso-

lated mitochondria, Hsu and Kummerow [17] found that the rate of ATP synthesis from oleoylcarnitine, erucoylcarnitine and elaidoylcarnitine decreased in this order, and Lawson and Kummerow [23] found that the oxidation rate of elaidoyl-CoA was lower than that of oleoyl-CoA or stearoyl-CoA. The oxidation rates of the latter two compounds were about the same.

As there are only a few data on rat heart, some data from hearts of other species are presented here. In myocardial homogenates and mitochondria isolated from guinea pigs [2] the ranking order in oxidation rate was: linoleic > oleic > palmitic acid. Mitochondria from beef hearts [18] oxidized 1-^{14}C linoleic acid faster than 1-^{14}C palmitic acid. However, for uniformly radioactive labelled fatty acids the ranking order of the oxidation rates was found to be: palmitic > oleic > linoleic > linolenic acid. An effect of the type of labelling on the results was also found by Stein and Stein [50]. However, Mathias *et al.* [25] did not find an effect of the type of labelling on the relative oxidation rates of fatty acids.

Although these data are again quite inconclusive, they support the finding obtained with perfused rat hearts that linoleic acid is oxidized at a somewhat faster rate than the other long-chain fatty acids.

1.3.5 Explanations for the differences in the oxidation rate of the various long-chain fatty acids

There are a number of possibilities to explain the differences in the oxidation rates of the various fatty acids. For oxidation, fatty acids must be set free from triacylglycerol, coupled to carnitine and transported into mitochondria. Coupling to carnitine is performed by carnitine acyltransferase of which there are 2 or 3 different ones with preference for medium- or long-chain fatty acids [3,20]. This type of enzyme has a low affinity for erucoyl-CoA [cf. 1.2.4.2] which shows that the rate of transport of fatty acids for oxidation can depend on the type of fatty acid.

Differences in the oxidation rates could also be caused by other influences. Long-chain acyl-CoA inhibits adenine nucleotide translocation in heart mitochondria [46,47], free fatty acids [22], palmitoylcarnitine and palmitoyl-CoA [27] inhibit Na^+,K^+-ATPase, and palmitoylcarnitine inhibits the calcium pump in cardiac sarcoplasmic reticulum [40]. It is not unlikely that the degree of inhibition observed is influenced by the type of the acyl moiety. This has already been shown for the inhibition of the adenine nucleotide translocation by CoA esters of long-chain fatty acids [4,24,39,45,47,53].

It is well known that free fatty acids can inhibit various enzymes. Whether this occurs in the myocardial cell will depend e.g. on the free fatty acid concentration. It has been found that the cellular free fatty acid concentration is influenced by the various types of fatty acid present in the triacylglycerols or

by those present in the perfusion fluid. Olson and Hoeschen [34] and Connellan and Masters [5] determined the fatty acid composition of the free fatty acid pool and of the triacylglycerols in the rat heart. They found that in the free fatty acid pool, in comparison with the amount in the triacylglycerols, the concentrations of palmitic, oleic and stearic acids were each relatively higher than that of linoleic acid. When hearts were perfused with linolenic or erucic acid the amount of the free fatty acids increased compared to perfusion with other long-chain fatty acids [41,54].

1.3.6 Conclusion

This review shows that in the heart there are differences in metabolism between the various long-chain fatty acids. It has been found that the oxidation rate of linoleic acid is faster than that of other long-chain fatty acids. Also differences have been found in inhibitory actions exerted on cellular processes by metabolites of the various long-chain fatty acids. Whether these differences affect the performance of the heart has to be investigated. It is likely that effects on the heart performance are not present in the normal, healthy heart. However, it has been found that during myocardial infarction the plasma free fatty acid level strongly increases [33], and that during myocardial hypoxia, ischemia or infarction the myocardial amounts of the CoA and carnitine esters of long-chain fatty acids rise [48,49,59]. It is posssible that in such circumstances differences in the metabolism of the various long-chain fatty acids or differences in inhibitory actions exerted by the various long-chain fatty acid esters of CoA and carnitine will lead to differences in cardiac performance.

It should be noted that the determination of the relative oxidation rates of long-chain fatty acids has been performed in the Langendorff-perfused heart, a preparation with a low utilization of substrate compared to the working heart [6,29]. In the working heart the differences in the utilization of different fatty acids could be more pronounced. Therefore, it would be of importance to investigate in the working heart the effects of perfusion with various fatty acids on external left ventricular work and on the energy charge by determining the adenine nucleotide and creatine phosphate levels in freeze-clamped heart or by NMR spectroscopy. Other methods such as surface fluorescence or determination of the adenosine, inosine and hypoxanthine release could also be indicative of the energy charge of the heart.

1.3.7 References

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2 METHODS AND PROCEDURES

2.1 THE ISOLATED PERFUSED HEART

2.1.1 Isolation of the heart

To isolate the hearts, rats and guinea pigs were anesthetized by intraperitoneal injection with Nembutal (sodium pentobarbital, 60 mg kg^{-1}) and rabbits by intravenous (ear vein) injection with Nembutal. Immediately after onset of the anesthesia the animals were heparinized (30 mg kg^{-1} intravenously), decapitated and the hearts isolated. The isolation of the rat and guinea pig hearts took about 1 min and the isolation of the rabbit heart about 2 min. Immediately after isolation, the hearts were perfused according to the conventional Langendorff technique.

2.1.2 Conventional Langendorff technique

A technique for isolated heart perfusion was first described by Langendorff in 1895 [22]. In this technique the heart is excised and the coronary vessels are perfused via the aorta with a buffer solution (Fig. 2.1). The heart hardly performs external work as the amount of fluid which is ejected by the left ventricle is very small and the pressure against which the right ventricle ejects the main part of the coronary venous effluent is zero. The fluid in the left ventricle comes mainly from arterio-luminal vessels and from leakage through the valves [28]. In the present study the heart was perfused immediately after excision at a perfusion pressure (P_p) of about 8.0 kPa* with a Krebs-Henseleit buffer solution [21] (NaCl, 118 mmol l^{-1}; KCl, 4.6 mmol l^{-1}; $CaCl_2.2H_2O$, 2.5 mmol l^{-1}; $MgSO_4.7H_2O$, 1.24 mmol l^{-1}; KH_2PO_4, 1.18 mmol l^{-1}; $NaHCO_3$, 25 mmol l^{-1}), supplemented with glucose (11.1 mmol l^{-1}) and ethylenediaminetetra-acetic acid (EDTA, 0.5 mmol l^{-1}), [28], and gassed with 95% O_2 + 5% CO_2 resulting in an oxygen tension of about 87 kPa. Temperature was 37.5 to 38 °C. This modified Krebs-Henseleit buffer will be designated KH buffer. The perfusion fluid was filtered over a Millipore® filter with a porosity of 3 μm. In a number of studies it has been shown that a glucose concentration of 11.1 mmol l^{-1} is sufficient as an external energy source (cf. 2.4).

*1 kPa = 7.5 mmHg

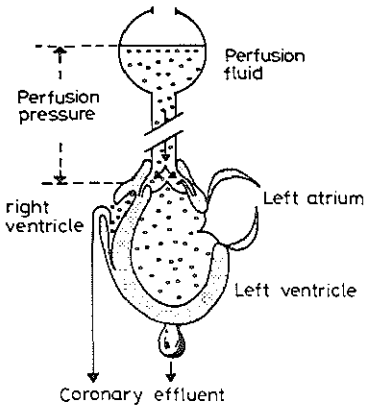

Fig. 2.1 The isolated, perfused heart according to Langendorff.

The amount of EDTA will bind about 0.5 mmol Ca^{2+} per l. Isolation of the heart induces an ischemic period of about 1 min causing a high coronary flow rate ($\dot{Q}_{cor}$) during the beginning of the perfusion. Therefore, generally, an adaptation time of about 10 min was used before the determinations of $\dot{Q}_{cor}$ and of the release of metabolites from the heart started.

2.1.3 Modified Langendorff technique

In the KH buffer-perfused heart, in comparison with the blood-perfused heart, the transcapillary flow rate via the capillary clefts will be higher because the viscosity of the KH buffer is low and the clefts are widened by the perfusion with this buffer [10, 37]. Since the KH buffer does not contain any colloids, there is no reabsorption of fluid through the capillary endothelium. So the interstitial fluid volume rises and, consequently, the lymphatic flow rate will increase. The lymphatic or interstitial effluent, also called transmyocardial effluent [42], can be separated from the coronary venous effluent by the technique described in Addendum I. Briefly, caval and pulmonary veins are tied off and the pulmonary artery is cannulated. The venous effluent is ejected by the right ventricle via the cannula, whereas the interstitial effluent drips from the heart. Due to the low flow rate of the interstitial effluent the concentration of compounds released by the heart will generally be much higher in the interstitial effluent than in the venous effluent or in the total coronary effluent. The latter is the sum of the interstitial and venous effluents. In the present study the interstitial effluent is mainly used for the determination of the release of prostacyclin (PGI_2) from the heart as the bioassay of PGI_2 used in the beginning of the study, was not sensitive enough to detect PGI_2 in total coronary effluent.

2.1.4 Isolated, working heart

The isolated, working rat heart has been described by Neely *et al.* [28] and is shown schematically in Fig. 2.2. Briefly, perfusion fluid is led to the left ventricle via the left atrium at left ventricular filling pressures (P_{lvf}) between 0.53 and 2.00 kPa (4-15 mmHg). The aorta is connected with a water column exerting a hydrostatic pressure of 9.3 kPa (except where mentioned otherwise).

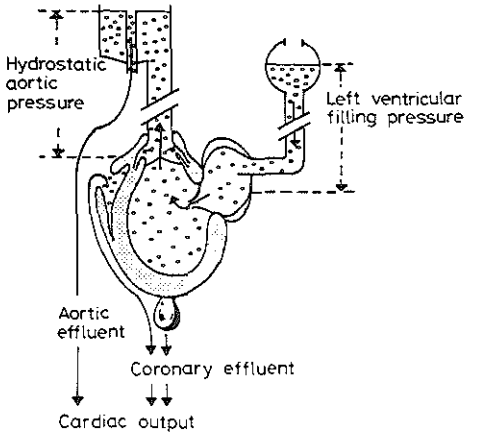

Fig. 2.2 The isolated, working heart.

The hydrostatic pressure is analogous to the diastolic aortic pressure in the intact animal and is designated hydrostatic aortic pressure (P_{aoh}). The working heart is used for the determination of the influence of the type of dietary fat on the external left ventricular work (W_{lv}). W_{lv} is calculated from the mean aortic pressure during ejection and the output of the left ventricle as described by Neely *et al.* [28] and Penpargkul and Scheuer [34] (cf. 2.5).

2.1.5 Perfusion apparatus

The perfusion apparatus which was usually used, is a modification of the perfusion apparatus described by Zimmerman [44], and is represented schematically in Fig. 2.3. The heart is connected to the perfusion apparatus using an aortic cannula made of an injection needle. Perfusion fluid (cf. 2.1.2) is stored in a 2 l reservoir (5) and oxygenated with 95% O_2 + 5% CO_2 in a 150 ml oxygenation chamber (10). The temperature of the perfusion fluid when entering the heart is 38 °C.

For Langendorff-perfusion the 3-way stop-cock (11) connects the oxygenation chamber with the heart. Vessel 5 and valve 12 are closed. Valve 7 is open and is connected to a tube immersed in a water column (9). By varying the length of the tube in the water column the gas pressure (P_g) in the oxygenation vessel can be varied. Perfusion pressure (P_p) is the sum of the hydrostatic pressure exerted by the water column above the heart (P_{wc}) and P_g in the oxygenation vessel.

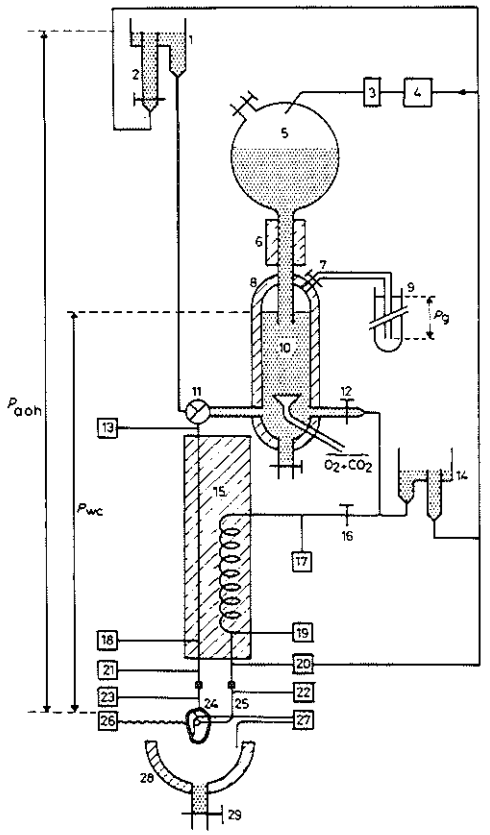

Fig. 2.3 Perfusion apparatus for the Langendorff-perfused and working heart. 1 and 14, overflow vessels; 2 and 29, graduated tubes; 3, Millipore® filter (porosity 3 μm); 4, roller pump; 5, reservoir; 6, 8 and 15, water jackets (38 °C); 7, 12 and 16, valves; 9, water column; 10, oxygenation vessel; 11, 3-way stop-cock; 13 and 17, Braun syringe pumps; 18 and 19, expansion vessels (10 ml) placed 7 cm above the heart; 20 and 27, oxygen-electrodes; 21, thermistor thermometer; 22 and 23, pressure transducers; 24, aortic cannula (inner diameter 0.21 cm, length 2 cm); 25, atrial cannula (inner diameter 0.16 cm, length 3 cm); 26, heart stimulator; 28, thermostated (38 °C) vessel. Between 1 and 11 siliconrubber tubing, inner diameter 0.4 cm; between 11 and 24 glass tubing, inner diameter 0.22 cm. From heart to fluid level in 5 (when filled) about 82 cm.

For the working heart valve 11 connects the heart with the overflow vessel 1; vessel 5 and valves 12 and 16 are open, valve 7 is closed. P_{lvf} can be regulated by adjusting the resistance of valve 12 and the height of the overflow vessel 14, and is measured in the atrial cannula (25) by a Statham P23V pressure transducer (22). The aortic pressure is measured in the aortic cannula (24) by a Statham P23Db pressure transducer (23), and using a Portex nylon cannula

(inner diameter 0.075 cm, length 10 cm) inserted through the aortic cannula to 2 mm above the opening. The left ventricular pressure is determined by a Statham P23Db pressure transducer connected by means of a nylon cannula (Portex, inner diameter 0.075 cm, length 6 cm) to a piece of a needle (inner diameter 0.035 cm, length 0.7 cm) inserted through the myocardial wall (not shown in Fig. 2.3).

In the Langendorff heart set-up the oxygen tension (P_{O_2}) of the perfusion fluid is measured just above the aortic cannula (not shown), and in the working heart set-up just above the atrial cannula. The P_{O_2} of the coronary venous effluent is measured using a cannula placed in the pulmonary artery. P_{O_2} is determined using Radiometer E 504 oxygen electrodes. In the modified Langendorff-perfused heart a cannula (inner diameter 0.16 cm. length 3 cm) is tied in the pulmonary artery (not shown in Fig. 2.3). The oxygen electrode (27) is connected to this cannula by means of a T-junction.

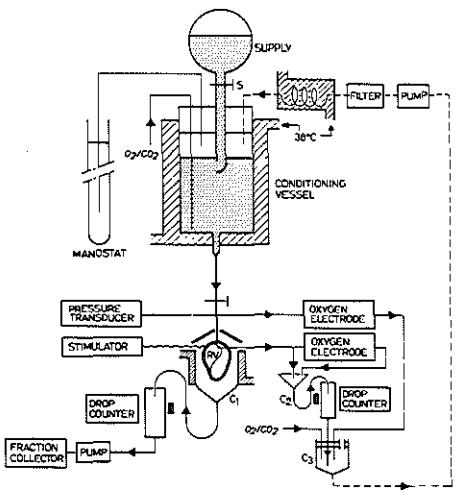

Fig. 2.4 Perfusion apparatus for metabolic studies using the modified Langendorff technique.

To recirculate the venous effluent from the modified Langendorff-perfused heart another type of perfusion apparatus was used. This is shown schematically in Fig. 2.4. For single-flow-through perfusion, the perfusion fluid runs from a 2 l supply vessel into a 0.2 l conditioning vessel where it is heated to 38 °C and gassed with O_2 / CO_2 (95 / 5). For recirculation of the venous effluent, valve S is closed and the venous effluent is collected in C_3, gassed with O_2 / CO_2 (95 / 5) and pumped through a filter (Millipore® ; diameter 47 mm; pore size 3 μm) and a heat exchanger into the conditioning vessel. In both types of

perfusion the venous effluent collected in the right ventricle (RV) is ejected through a cannula (inner diameter 0.16 cm, length 3 cm) tied in the pulmonary artery. The cannula is connected with a T-junction transferring the venous effluent partly directly into C_2 and partly along a second oxygen electrode. The interstitial effluent, dripping from the heart, is collected in C_1 and pumped towards a fraction collector using Portex PP50 tubing. The flow rates of the venous and interstitial effluents are measured by counting the drops from C_1 and C_2 (cf. Fig. 2.5) using a photo-electrical drop counter made in our own workshop.

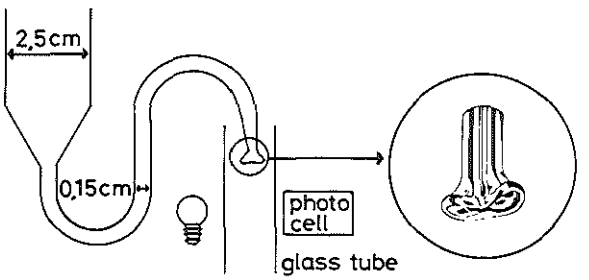

Fig. 2.5 Vessel used to measure the flow rate of effluent from the isolated heart; saddle-shaped out-flow opening [40] is shown in detail.

The perfusion set-up was always rinsed with KH buffer before use. The first sample of the interstitial effluent, collected for 5 min, was always discarded. P_p is measured just above the aortic valve using a Statham P23Db pressure transducer. The oxygen tensions of the perfusion fluid and the venous effluent are measured using Radiometer E 504 oxygen electrodes.
In both perfusion set-ups, hearts were stimulated electrically (by two electrodes placed on the right atrium) at a pacing frequency (f_p) of 360 min^{-1} (10 V, duration 3 ms, rectangular pulses), except where mentioned otherwise. A stimulator made in our own workshop was used.

2.1.6 Determination of heart parameters

Coronary flow rate ($\dot{Q}_{cor}$) is determined by measuring the time needed to collect 5 ml of the total coronary effluent in a graduated tube (number 29 in Fig. 2.3). To determine $\dot{Q}_{cor}$ every min the total coronary effluent is collected in a small vessel with a special drop forming device (Fig. 2.5), which is placed under the heart. The number of drops per min is counted by a photo-electrical device. Due to the drop forming device the drop volume is only to a very small extent dependent on the number of drops per min (Fig. 2.6). For the determination of this relationship KH buffer (37.5 °C) was used. The collected

volume (per min) was determined by weighing, dividing the mass by the density of KH buffer at 37.5 °C.
Myocardial oxygen consumption ($\dot{V}_{O_2}^m$) is calculated from $\dot{Q}_{cor}$ and the difference in the oxygen tension between the perfusion fluid entering the heart and the venous effluent [28].

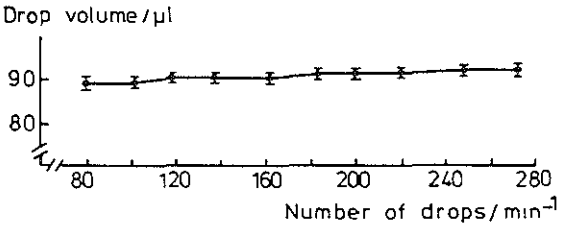

Fig. 2.6 Relationship between drop volume and number of drops obtained with the saddle-shaped outflow opening (cf. Fig. 2.5). Mean values (± SD) of 5 determinations.

Output of the heart ($\dot{Q}_{lv}$) is the sum of $\dot{Q}_{cor}$ and the flow rate of the aortic effluent. The aortic effluent is the fluid ejected by the left ventricle via the overflow vessel (number 1 in Fig. 2.3), and is determined by measuring the time needed to collect 20 ml in a graduated tube (number 2 in Fig. 2.3).
Mean aortic pressure ($\bar{P}_{ao}$) is approximated using the equation

$$\bar{P}_{ao} = P_{aoh} + \frac{P_{aop} - P_{aoh}}{2} \qquad (2.1)$$

where P_{aoh} is the hydrostatic aortic pressure and P_{aop} is the peak aortic pressure during ejection. P_{aop} (measured in the aortic cannula) is almost equal to the peak value of the left ventricular systolic pressure (measured using a thin needle through the myocardial wall). This is shown in Fig. 2.7 at 2 values of P_{lvf}. For 5 hearts the difference between P_{aop} and the peak value of the left ventricular systolic pressure was 0.3 ± 0.1 (SD) kPa at P_{lvf} = 1.33 kPa.
For the calculation of W_{lv} from $\bar{P}_{ao}$ and $\dot{Q}_{lv}$ see section 2.5.
Spontaneous heart frequency (f_s) is measured by counting the pulsations in the aortic pressure. Usually f_s is measured at about 10 min after the start of perfusion.
Dry heart mass (m_h) is obtained by drying the heart at 80 °C for 24 h.

2.1.7 Perfusion of hearts with various compounds

To perfuse the isolated heart with various compounds, these were dissolved in a vehicle and infused into the perfusion fluid above the heart (Fig. 2.3) at such a rate that the dilution by the perfusion fluid was at least 200 times (cf. Ad-

denda VI and VII). Stock solutions of the fatty acids (purity 98%) and prostaglandins, except PGI_2, were made in ethanol and kept at –20 °C under nitrogen. A stock solution of the sodium salt of PGI_2 ($NaPGI_2$) was stored in NaOH (0.1 mol l^{-1}) at 4 °C. For infusions, fatty acids were dissolved in saline containing NaOH (1.5 mmol l^{-1}) under nitrogen. Prostaglandins, except PGI_2, were dissolved in KH buffer. $NaPGI_2$ stock solution was diluted with saline containing NaOH (2 mmol l^{-1}). Indomethacin was dissolved in ethanol (55 mmol l^{-1}) and diluted with KH buffer.
Arachidonic acid and the prostaglandins were synthesized by the Chemical and Biochemical Departments of the Unilever Research Laboratorium at Vlaardingen. Indomethacin was obtained from Merck, Sharp and Dohme, Haarlem, The Netherlands.

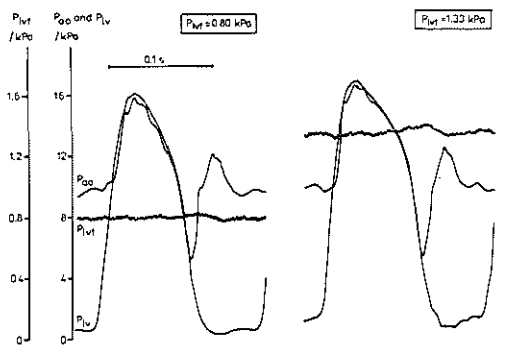

Fig. 2.7 Isolated, working heart. Pressures measured in the left ventricle (P_{lv} and in the aortic cannula (P_{ao}) at two values of the left ventricular filling pressure (P_{lvf}).

2.1.8 Determination of the relationship between heart frequency and maximal external left ventricular work

In the isolated, working rat heart preparation the sinus node was removed by cutting away the part of the right atrium between the superior (right) and inferior vena cava. As it was found (cf. 2.5) that maximal external left ventricular work ($W_{lv}(max)$) at $P_{aoh} = 9.3$ kPa is obtained at a value of $P_{lvf} \approx 1.33$ kPa, P_{lvf} was kept at this value. Fifteen min after the start of perfusion, the hearts were stimulated at a frequency of 320 min^{-1} and every 4 min f_p was increased by 10 min^{-1} to 400 min^{-1}. W_{lv} for $P_{lvf} = 1.33$ kPa was determined at each value of f_p. In a number of hearts f_p was then lowered from 400 min^{-1} to 360 min^{-1} to determine whether an observed decrease in W_{lv} at $f_p > 360$ min^{-1} was due to deterioration of the heart.

2.1.9 Metabolic studies using the modified Langendorff technique

Glucose uptake and lactate and fatty acid release in the isolated rat heart were determined using the modified Langendorff technique. A schematic representation of the perfusion set-up used is given in Fig. 2.4. After isolation, the heart was prepared for perfusion according to the modified Langendorff technique. Fifteen min after the isolation of the heart, the interstitial effluent was collected in 3 periods of 10 min. Glucose and lactate were determined in the perfusion fluid and in the 3 collected samples; free fatty acids were determined in the perfusion fluid and in a pooled sample of the 3 collected samples. The changes in the concentrations of glucose and lactate in the interstitial effluent accounted for 80 to 90% of the glucose uptake and lactate release by the heart (Addendum I). The remaining 10 to 20% can be calculated from the changes in the concentrations in the venous effluent. As it was a comparison between two groups of hearts, this amount was not taken into account and the venous effluent was discarded.

For the determination of the release of prostaglandins from the heart into the interstitial and venous effluents (using the perfusion set-up shown in Fig. 2.3) the heart was adapted to the perfusion conditions for 20 min. Next, the interstitial effluent was collected directly into a tube surrounded by ice for a period of 10-15 min. The venous effluent was also collected for a period of 10-15 min.

2.2 OTHER PROCEDURES

2.2.1 Determination of prostaglandins

2.2.1.1 Gas chromatography

The gas chromatographic determination of prostaglandins has been described in Addendum II. Briefly, to perfusates ω-nor-PGE_2 and $PGF_{1\alpha}$ were added as internal standards. $PGF_{1\alpha}$ was used as internal standard for 6-oxo-$PGF_{1\alpha}$ as the standard ω-homo-6-oxo-$PGF_{1\alpha}$ was not yet available at that time. The perfusates were acidified (pH 4.0) and extracted twice with ethylacetate. The extract was vacuum-dried. The prostaglandins in the extract were purified by thin-layer chromatography as their methoximated pentafluorobenzyl esters and after silylation determined by gas chromatography with electron-capture detection.

It was later found (DH. Nugteren, personal communication) that the yield of 6-oxo-$PGF_{1\alpha}$ after isolation and derivatisation was 2 to 3 times less than that of $PGF_{1\alpha}$. The originally found values of 6-oxo-$PGF_{1\alpha}$ were multiplied by a factor of 2.5 in Addendum VII, but not in Addenda II and V.

2.2.1.2 PGI_2-like substance

PGI_2-like activity of perfusates was determined by measuring the inhibitory activity of a sample of perfusate on ADP-induced rat platelet aggregation [20] using a modified Vitatron photometer or a Lumi-aggregometer (Chrono-log Corp.). The activity was considered to be due to a PGI_2-like substance when the inhibition disappeared after standing of the test sample at room temperature for 20 min [8,16]. Initially, PGI_2-like substance was determined in the interstitial effluent, as only in the interstitial effluent the concentration of PGI_2-like substance was high enough for detection with the method in which the test sample had to be diluted to avoid clotting of the platelets due to the KH buffer.The procedure is described in Addendum II, see especially Fig. 2. PGI_2-like substance was determined in total coronary effluent without dilution after two changes in the procedure. Clotting of the platelets caused by KH buffer was prevented by adding heparin (5 mg l^{-1}) to the perfusion fluid. Since the dose-response curve of the relationship between the concentration of PGE_1 or PGI_2 and the inhibition of the ADP-induced platelet aggregation is S-shaped [20], the low amount of PGI_2-like substance usually present in the coronary effluent of the classical Langendorff-perfused heart cannot be detected. However, the sensitivity of the method can be increased by measuring in the steep part of the S-shaped curve. In order to achieve this an extra amount of PGI_2 (or PGE_1) was added to the incubation mixture (see below), through which the sensitivity of the method for PGI_2 detection became about 0.15 nmol l^{-1}.

The procedure using the modified Vitatron photometer is as follows; 2.4 ml of a sample of coronary effluent, 0.3 ml platelet-rich plasma and 0.1 ml of a NaPGI$_2$ solution (2.6 nmol l^{-1}) in NaOH (2 mmol l^{-1}) were incubated at 37 °C; after 2 min, 0.2 ml of an ADP solution (3.2 nmol l^{-1}) was added and the change in light transmission of the incubation mixture was measured. The degree of inhibition by samples of coronary effluent was measured against inhibition by PGI_2. The procedure using the Lumi-aggregometer is similar; this is described in Addendum VII.

2.2.2 Determination of respiratory properties of isolated heart mitochondria

Heart mitochondria were isolated as described by Hülsmann [18]. One tenth ml of mitochondrial suspension was diluted with 1.6 ml of a buffer solution of pH 7.4 containing KH_2PO_4 (25 mmol l^{-1}), KCl (50 mmol l^{-1}), tris-hydroxymethyl-aminomethane (25 mmol l^{-1}), $MgCl_2$ (2.5 mmol l^{-1}), EDTA (0.5 mmol^{-1}), ATP (0.5 mmol l^{-1}) and bovine albumin, 0.032%, and incubated at 35 °C in a Gilson oxygraph (model KM), provided with a micro Clark electrode. To the incubation medium 0.1 ml of a mixture of a glutamate (200 mmol l^{-1}) and malate (20 mmol l^{-1}) solution was added. Oxygen consumption (Q_{O_2}) was determined before and after the addition of 0.6 μmoles ADP (in 0.02 ml).

Q_{O_2} is expressed in moles of O_2 consumed per mg protein per min. Protein content of the mitochondrial suspension was analysed by the biuret method.

2.2.3 Determination of myosin ATPase activity

The determination of the myosin ATPase activity was performed as described by Bhan and Scheuer [4]. Hearts were homogenized, and after centrifugation, the residue was further homogenized in a solution of Triton X-100 and centrifuged. The residue obtained (largely myofibrils) was extracted with a solution of KCl and pyrophosphate. After centrifugation, the myofibrils in the supernatant were precipitated, dissolved again and the solution was again centrifuged. The supernatant, now containing the myosin, was fractionated with ammonium sulfate. The myosin precipitated was dialyzed until free of ammonium sulfate. Myosin ATPase activity was determined by adding ATP to a solution of the myosin and measuring the ADP formed with the Biochemica Test Combination (Boehringer, Mannheim) for the determination of ADP and AMP.

2.2.4 Determination of glucose, lactate, free fatty acids and the fatty acid composition of phospholipids

All determinations were performed according to standard procedures. Glucose was assayed with glucose oxydase (Beckman Glucose Analyzer). Lactate was assayed with lactate dehydrogenase and NAD^+ (Biochemica Test Combination, Boehringer, Mannheim). The total fatty acid composition of mitochondria was determined by GLC [45] after extraction [5] and separation of the lipid classes by TLC. The fatty acids in perfusates were determined by a photometric method as described by Laurell and Tibbling [23]. The phospholipid fatty acid composition of myocardial tissue was determined in pooled samples of ventricular tissue taken from the heart immediately after perfusion and frozen in liquid nitrogen. The lipids were extracted as described by Bligh and Dyer [5], the lipid classes separated by TLC and the fatty acids determined by GLC [45].

2.2.5 Determination of myocardial Na^+, K^+-ATPase activity

Myocardial Na^+,K^+-ATPase activity was determined by measuring the ouabain-sensitive uptake of Rb^+ by ventricular tissue slices as described by Curfman *et al.* [12]. The Rb^+ uptake is determined instead of the K^+ uptake because a suitable radioactive isotope is available of this ion, which is also transported by the Na^+,K^+-ATPase (The Radiochemical Centre, Amersham, UK; specific activity 0.61 Ci $mmol^{-1}$). After isolation, hearts were perfused with 10 ml KH buffer (0 °C) after which the middle part of each heart was cut into 6 ventricular slices (approximately 0.5 mm thick) which were washed in KH buffer (0 °C) for a few minutes. Three randomly chosen slices of each

heart were incubated in 10 ml KH buffer supplemented with RbCl (0.1 mmol l^{-1}) containing $^{86}Rb^+$ (0.8 mCi l^{-1}). The other three slices were incubated in the same incubation medium to which ouabain (0.1 mmol l^{-1}) was added to inhibit the Na^+,K^+-ATPase activity. The incubation media had been oxygenated with 95% O_2 + 5% CO_2 and this gas mixture was also present above the incubation mixture. The slices were incubated in a shaker for 30 min at 30 °C. Hereafter, the slices were washed with KH buffer (0 °C) for 30 s, blotted and weighed. Slices were then put into 10 ml KH buffer + 0.1 $mmol^{-1}$ RbCl, and tissue radioactivity was assayed using a Packard gamma scintillation spectrometer. Values obtained from the slices containing added ouabain were subtracted from those obtained from slices without ouabain to calculate the $^{86}Rb^+$ uptake due to cation pump activity. The ouabain-sensitive $^{86}Rb^+$ uptake was approximately 35% of the total uptake in control slices.

2.2.6 Isolated perfused aorta

A piece of thoracic aorta (length about 3 cm) was isolated, rinsed and perfused (5 ml min^{-1}) with KH buffer (37 °C) for 9 min. The perfusion set-up is schematically shown in Fig. 2.8. After the equilibration period the release of PGI_2-like substance was found to be constant for 30 min. To increase the concentration of PGI_2-like substance in the perfusate, the flow rate was lowered to 0.375 ml min^{-1}. Perfusates were collected on ice for periods of 4 min and tested for PGI_2-like substance, using the blood platelet bioassay (cf. *2.2.1.2*).

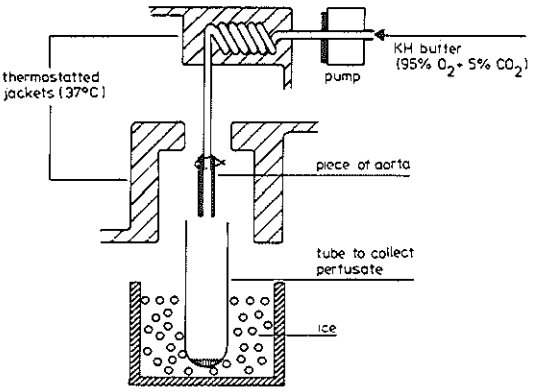

Fig. 2.8 Perfusion set-up for the isolated perfused aorta.

2.3 ANIMALS, HOUSING AND DIETS

2.3.1 Animals and housing

Male, specific pathogen free (SPF) Wistar rats of the CPB-WU strain were obtained as 20 to 22-day-old animals from the Central Institute for the Breed-

ing of Laboratory Animals TNO, Zeist, The Netherlands. Before the start of the experimental feeding period they were housed in stainless steel, wire bottomed cages (68 x 27 x 17 cm) in groups of about 7 animals. The cages were placed in air conditioned rooms (23 ± 1 °C) with a relative humidity of about 55%. A fixed day-night rhythm was applied (light from 7 to 19 h).
For dietary experiments, rats of the same lot number were used and groups on the basis of matching body mass were composed. At the start of the experimental diets the rats were 3 to 6-month-old. During the experimental feeding period rats were housed individually in stainless steel, wire-bottomed cages of 17 x 27 x 17 cm.
Male, Dutch rabbits were obtained from the Broekman Institute (Stiphout, The Netherlands) as 3-month-old animals (body mass about 1000 g). They were housed individually in cages of 62 x 30 x 30 cm. One week after delivery, groups with the same body mass distribution were composed and fed the experimental diets.
Male, guinea pigs were obtained as 7-week-old animals from the Central Institute for the Breeding of Laboratory Animals TNO. They were housed in groups of 5 animals in plastic boxes (80 x 50 x 40 cm) containing straw.

2.3.2 Diets

Before feeding experimental diets, rats and rabbits were fed commercial laboratory diets, "Muracon" obtained from Trouw and Co., Putten, and "Konijnenkorrel" obtained from UTD, Maarssen, The Netherlands, respectively. Guinea pigs were fed "Cavicon" (Trouw and Co.). The commercial diets contained about 24% of the digestible energy (en%) as protein, and 63-68 en% carbohydrates. "Muracon" and "Cavicon" contained about 13 en% and "Konijnenkorrel" 8 en% fat. According to the manufacturers, the diets contained sufficient amounts of vitamins, minerals and linoleic acid. In a number of samples the amount of linoleic acid was determined. In "Muracon" this was about 6 en% and in "Konijnenkorrel" 3.3 en%.
During the experimental feeding period the animals were fed semi-synthetic diets supplemented with 20 g sterilized saw dust per 4184 kJ (1000 kcal). The diets contained 23 en% protein (casein for rats; soyabean protein for rabbits). This amount is sufficient to meet the protein requirement of growing rats and rabbits [9,27]. The fat amount varied between 25 and 50 en%. The remaining amount of the energy was supplied as corn starch. To all the diets a sufficient amount of minerals and vitamins was added. For rats the mineral and vitamin mixtures added are described by Vles *et al.* [39]. To the diets of rabbits more potassium was added [38]. The animals had free access to food and water.
The fatty acid composition of the fats used in the experimental diets are listed in Table 2.1. The amounts of fatty acids are expressed in % (w / w). To calculate the amount of a fatty acid in en%, the percentage of the fatty acid in the

fat is multiplied by the amount (en%) of the fat in the diet. In some experiments (Addendum III) diets containing 50 en% fat were fed, assuming that this high amount would induce a rapid effect. However, in most experiments diets containing 35 en% fat were supplied as this amount is more in agreement with the desired amount of fat in the human diet.

TABLE 2.1 Main fatty acid composition (%, w/w) of the dietary fats used in the experiments.

Fatty acid	SSO	SBO	OO	LSO	PO	L	HSBO	HCO	TLG	TPG
C 8:0								7.4		
C 10:0								5.6		
C 12:0								45.3		
C 14:0	0.1				1.0	2.5		17.9		
C 16:0	6.6	10.9	12.7	6.3	44.1	28.3	11.6	9.4		98
C 16:1 (n-7)	0.1	0.1	1.4	0.1	0.2	3.2	0.1			
C 18:0	4.5	4.0	2.4	5.2	4.7	13.7	7.8	13.6		
C 18: 1(n-9)	15.8	22.8	71.1	19.0	38.7	40.3	71.6[1]			
C 18:2 (n-6)	70.5	53.6	11.1	16.1	10.0	9.1	7.2[2]		98	
C 18:3 (n-3)	0.2	7.6	0.6	51.5	0.3	0.7				
C 20:0	0.3	0.6	0.3		0.6	0.1	0.4			
C 20:1 (n-9)	0.2	0.3	0.2	0.5	0.3	0.8	0.2			
C 22:0	1.1	0.3		0.4			0.4			

SSO, sunflowerseed oil; SBO, soyabean oil; OO, olive oil; LSO, linseed oil; PO, palm oil; L, lard; HSBO, hydrogenated soyabean oil [19, HSBO-3]; HCO, hydrogenated coconut oil; TLG, trilinoleoylglycerol; TPG, tripalmitoylglycerol. TLG and TPG were artificial fats and contained about 2% of other fatty acids, mainly oleic acid.

[1]) About 51% (w/w) (*E*) C 18:1 (elaidic acid)

[2]) (*E.Z.*)/(*Z.E.*) C 18:2

Dietary groups will be indicated by the abbreviations of the fats and the amounts (in en%) of the fats used in the experimental diet. For instance dietary group 33 L + 2 SSO means that the rats were fed a diet containing 33 en% L and 2 en% SSO.

2.4 CONSIDERATIONS ON THE USE OF THE ISOLATED, WORKING RAT HEART FOR PHYSIOLOGICAL INVESTIGATIONS

Isolated, perfused heart preparations are useful for many types of investigation, because they permit the accurate determination of many quantities such as oxygen consumption, external work, coronary flow rate, uptake and release of various compounds, and exogenous and endogenous substrate consumption. The preparations should be stable over a reasonably long perfusion period and the values of the parameters determined comparable to the values obtained in the intact animal. Numerous investigations have shown that the Langendorff heart can be stable for at least 1 h. For instance, recent studies in the Langendorff-perfused rat [2, 29] and guinea pig heart [6] show that the

myocardial concentrations of creatine phosphate and adenine nucleotides are quite constant for at least 1 h of perfusion. Also in the working rat heart it was found that the concentrations of these compounds are rather constant [30, 32]. The isolated, working heart is a recently developed technique [28], and much less information is available about the mechanical stability of this preparation. Neely *et al.* [28] have shown that at $P_{lvf} = 1.0$ kPa, W_{lv} and $\dot{V}^{m}_{O_2}$ were stable for 3 h of perfusion. Progressive deterioration of mechanical function was observed during the 4th hour of perfusion. At $P_{lvf} = 2.0$ kPa, W_{lv} was only stable for 1 to 1.5 h. Penpargkul and Scheuer [34] and Crass III *et al.* [11] found $\dot{Q}_{cor}$, $\dot{V}^{m}_{O_2}$ and W_{lv} stable for 1 h of perfusion. We found that mechanical performance was stable during the time of our measurements (usually about 45 min). This is demonstrated by Fig. 2.9 which shows 2 similar left ventricular function curves obtained one after the other. This figure shows that a duplicate measurement can be carried out with excellent results. However, when the left ventricular function curve was determined a third time, the reproducibility was bad, especially at higher values of P_{lvf}.
The stability of the mechanical performance of the heart can be affected by the formation of edema. However, in the rat heart perfused with KH buffer, edema hardly occurs [2, 41].

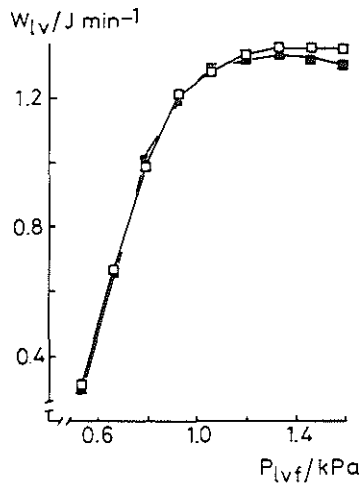

Fig. 2.9 Reproducibility of the determination of the relationship between left ventricular filling pressure (P_{lvf}) and external left ventricular work (W_{lv}).
The first function curve (□——□) was determined from 10 to 25 min after the start of perfusion, after which P_{lvf} was again decreased to 0.53 kPa and the second function curve (■——■) was determined. Mean values of 2 hearts. Mean value of dry heart mass: 224 mg.

The values of W_{lv} and $\dot{V}^{m}_{O_2}$ obtained in our experiments are comparable with those obtained in the intact animal and in experiments in which the isolated heart was perfused with whole blood or KH buffer to which red blood cells and albumin had been added (Tables 2.2 and 2.3). In the blood-perfused heart it was found that $\dot{V}^{m}_{O_2}$ was linearly related to the left ventricular work load [14]. Also in our experiments it was found that $\dot{V}^{m}_{O_2}$ (and also $\dot{Q}_{cor}$) was linearly related to W_{lv} (Addendum III, Figs. 1 and 2). Two other arguments for the adequacy of the oxygenation of the working rat heart perfused with KH buffer are that the venous P_{O_2} values ranged from 20 to 40 kPa and that when hearts were perfused with epinephrine, W_{lv}(max) rose, e.g. to 1.8 J min^{-1}, $\dot{Q}_{cor}$ rose to 30 ml min^{-1} and $\dot{V}^{m}_{O_2}$ to 18 μmol min^{-1} (m_h = 220 mg), indicating that the heart can respond to an increased O_2 demand with a further increase in $\dot{Q}_{cor}$.

When hearts are perfused with buffer solutions without added erythrocytes, $\dot{Q}_{cor}$ is always much higher than in the intact animal where $\dot{Q}_{cor}$ is about 5% of the cardiac output. This percentage was also obtained when working hearts were perfused with KH buffer to which erythrocytes and albumin had been added [13] or with whole blood [14]. In both experimental situations $\dot{Q}_{cor}$ was between 1 and 6 ml min^{-1}. The high value of $\dot{Q}_{cor}$ in perfusions with buffer solutions is not only due to the low oxygen capacity of the buffer solutions but also to the low viscosity of the buffer [1, 41, 43]. Other factors like prostaglandin synthesis (cf. Addendum VII) and type of exogenous substrate [6] also affect $\dot{Q}_{cor}$.

The external substrate supply is of great importance for the stability and reliability of the isolated rat heart. In all our experiments 11.1 mmol glucose per l was used. The following brief survey indicates that the external energy supply becomes insufficient below a glucose concentration of 10 mmol l^{-1}. In isolated, working hearts and in Langendorff hearts, Bünger *et al.* [6, 7] found that by perfusion with 5.5 mmol glucose per l, the hearts deteriorated slowly, but that they were stable when perfused with 5.5 mmol glucose + 2 mmol pyruvate per l. Crass III *et al.* [11] found that in hearts perfused with 5.0 mmol glucose per l, the amount of triacylglycerol decreased but that it remained constant or even increased during perfusion with palmitate (1.0 mmol l^{-1}). However, Oram *et al.* [33] found that at high levels of cardiac activity the oxidation of exogenous palmitate, in contrast to that of octanoate, was not fast enough to maintain a high level of acetyl-CoA. Probably 5.0 mmol glucose per l does not supply enough energy when insulin is absent. In Langendorff hearts perfused with 8.3 mmol glucose per l, Gartner and Vahouny [15] did not find a decrease in the triacylglycerol content. Opie *et al.* [31] found in Langendorff hearts that the amount of glucose converted into CO_2 increased when the glucose concentration of the perfusate was increased from 5 to 10

TABLE 2.2 Values of external left ventricular work (W_{lv}) obtained in different rat heart preparations.

Method	Perfusion fluid	Ca^{2+} /mmol l^{-1}	Experimental conditions				W_{lv} /J min^{-1} g^{-1}	Reference
			f /min^{-1}	$\bar{P}_{ao}$ /kPa	P_{aoh} /kPa	P_{lved};P_{lvf} /kPa		
Anesthetized rats	Blood		360	17.3		2.0	1.12	24
			460	16.0		—[1])	1.04	3
Heart lung preparation	Blood		330	—[1])		2.0	0.64	17
Isolated, working heart	KH buffer + erythrocytes	2.5	280		9.3	1.7	0.80	13
	KH buffer	2.5	240		6.8	2.0	0.78	28
		2.0	325		8.3	2.0	0.76	34
		2.5	360		9.3	1.3	1.20	This study

The data from the literature were recalculated in SI units. For W_{lv} values (expressed per g wet heart mass), maximum values or the highest values reported by the authors, are shown. As in all cases, left ventricular end-diastolic pressure (P_{lved}) or left ventricular filling pressure (P_{lvf}) were quite high, it can be assumed that these highest values of W_{lv} were the maximal or near-maximal values of W_{lv} obtained under the experimental circumstances.

f, heart frequency; $\bar{P}_{ao}$ mean aortic pressure; P_{aoh} hydrostatic aortic pressure.

[1]) No data available.

mmol l^{-1}, but did not increase further at higher concentrations. Morgan *et al.* [26] and Post *et al.* [35] found that the rate of glucose uptake did not increase further above 11.0 mmol glucose per l. Studies in isolated, working hearts from normal and diabetic rats by Miller [25] have shown that the glucose-6-phosphate, glycogen and ATP contents were about the same at 10 and 30 mmol glucose per l in the perfusate and higher than at 5 mmol glucose per l. Moreover, in the diabetic heart glucose uptake was impaired at 5 mmol l^{-1} but not at 10 mmol l^{-1}.

TABLE 2.3 Myocardial oxygen consumption ($\dot{V}^m_{O_2}$) of the isolated rat heart at high perfusion pressure (P_p) and high left ventricular filling pressure (P_{lvf}).

Method	Perfusion fluid	P_p /kPa	P_{lvf} /kPa	$\dot{V}^m_{O_2}$ /mol $min^{-1}g^{-1}$	Reference
Langendorff perfusion	Whole blood	21	—	9.0 - 14.4	14
Working heart[1])	KH buffer + erythrocytes	—	1.3	13.4 - 15.6	13
Working heart[1])	KH buffer	—	1.3	12.4 - 14.0	This study

The values of $\dot{V}^m_{O_2}$ from the literature were recalculated in μmol min^{-1} per gram of wet heart mass.

[1]) For experimental conditions see Table 2.2.

2.5 DETERMINATION OF EXTERNAL LEFT VENTRICULAR WORK AND CARDIAC CONTRACTILITY

The effect of dietary fat on the mechanical performance of the heart was studied in the isolated, working rat heart. In this heart preparation the relationship between left ventricular filling pressure (P_{lvf}) and external left ventricular work (W_{lv}) was determined. The graph of this relationship, the left ventricular function curve, is S-shaped [36] (Figs. 2.9 and 2.10). Usually only the upper part of the graph was determined, since the maximal value of W_{lv} (W_{lv}(max)) was used as parameter to compare the effect of dietary fats on the mechanical performance of the heart. W_{lv} expressed in J min^{-1} was calculated as pressure-volume "minute work" from the equation:

$$W_{lv} = \dot{Q}_{lv} \cdot (\bar{P}_{ao} - P_{lvf}) \cdot 10^{-3} \qquad (2.2)$$

where $\dot{Q}_{lv}$ is the total left ventricular output (ml min^{-1}), $\bar{P}_{ao}$ is the mean aortic pressure (kPa) and P_{lvf} is the left ventricular filling pressure (kPa).

Kinetic work of the left ventricle (W_{lvk}) expressed in J min^{-1} was calculated from the equation:

$$W_{lvk} = \dot{Q}_{lv} \cdot \rho_p \cdot \frac{\dot{Q}_{lv}}{A_{ao}} \cdot 1.4 \cdot 10^{-11} \qquad (2.3)$$

where $\dot{Q}_{lv}$ is the left ventricular output (ml min^{-1}), ρ_p is the specific density of the perfusate at 37 °C in g ml^{-1} and A_{ao} is the cross-sectional area of the aorta in cm^2; ρ_p was 1.002 g ml^{-1} and for A_{ao} the cross-sectional area of the aortic cannula (0.035 cm^2) was taken.

W_{lvk} is small when compared to W_{lv}. For instance in the experiment shown in Fig. 2.10 (no epinephrine), $W_{lv}(max)$ = 1.32 J min^{-1} and $\dot{Q}_{lv}$ = 102 ml min^{-1}.

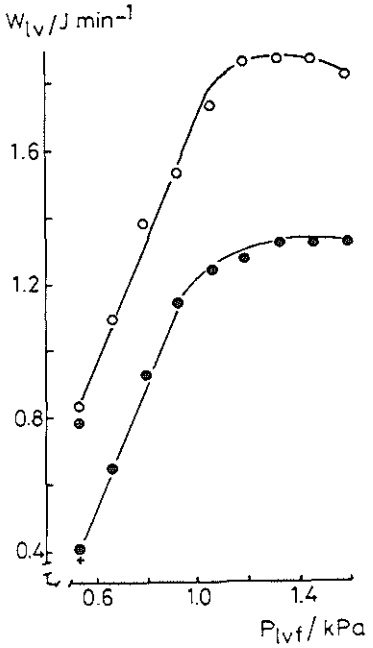

Fig. 2.10 Relationship between left ventricular filling pressure (P_{lvf}) and external left ventricular work (W_{lv}) in the working rat heart before and during perfusion with epinephrine.
P_{lvf} was increased every 2 min and W_{lv} determined (●——●). Next, P_{lvf} was decreased to the starting value of 0.53 kPa and W_{lv} determined (+), after which the heart was perfused with epinephrine (27 mmol l^{-1}). The relationship between P_{lvf} and W_{lv} was determined again (○——○). Next, P_{lvf} was again decreased to 0.53 kPa and W_{lv} determined (⊕). Determination in 1 heart; dry heart mass: 206 mg.

From Equation (2.3) it follows that $W_{lvk} = 0.012$ J min^{-1}. With epinephrine in the perfusate $W_{lv}(max) = 1.87$ J min^{-1} and $\dot{Q}_{lv} = 123$ ml min^{-1}. From Equation (2.3) it then follows that $W_{lvk} = 0.021$ J min^{-1}. These calculations show that W_{lvk} is only about 1% of W_{lv}. Therefore, in all calculations of the external left ventricular work W_{lvk} was neglected.

The fundamental relationship between muscle performance and initial fibre length forms the basis of the observed relationship between P_{lvf} and W_{lv}. Here P_{lvf} is taken as a measure of ventricular end-diastolic volume, which is in turn related to the initial myocardial fibre length. Obviously, P_{lvf} is an unreliable parameter for muscle fibre length, since the compliance and shape of the ventricle affect the relation between P_{lvf} and muscle fibre length. Consequently, when feeding different dietary fats leads to differences in the relationship between P_{lvf} and W_{lv}, then both contractility and compliance can have changed: changes in contractility will result in upward or downward shifts of the function curves with respect to each other; changes in compliance will affect fibre length at constant P_{lvf} as a result of which shifts of the function curves to the right or left occur. When it is impossible to make ventricular function curves of the same heart under various conditions - as was the case in the present study - it is difficult to tell whether an upward (downward) shift or a left (right) shift has occurred. To distinguish between the two conditions, we resorted to the assumption that in the case of a change in contractility $W_{lv}(max)$ will be obtained at the same value of P_{lvf}, whereas when the compliance has changed $W_{lv}(max)$ is found at different values of P_{lvf}. Consequently, when $W_{lv}(max)$ had increased at constant P_{lvf} this was interpreted as an increase in contractility, analogous to the increase in $W_{lv}(max)$ evoked by epinephrine (Fig. 2.10).

2.6 STATISTICS

The statistical evaluation of the results was carried out by the analysis of variance, the one-way or two-way analysis of covariance, Newman-Keuls test and Student's t test for paired or unpaired data. Usually the type of test used will be mentioned, except Student's t test. A special effect was assumed to occur, when the level of significance for such an effect exceeded 95% ($P < 0.05$). In those cases in which it was evident that statistically significant differences did not exist, statistical evaluation was omitted.

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3 RELATIONSHIP BETWEEN DRY HEART MASS AND MAXIMAL EXTERNAL LEFT VENTRICULAR WORK AND CORONARY FLOW RATE; EFFECT OF DIETARY FAT

3.1 INTRODUCTION

It has been found by Neely *et al.* [3] that in the working rat heart, external left ventricular work (W_{lv}) expressed per g of dry heart mass (m_h) decreases with an increase in m_h. In their experiments, the heart frequency was not kept constant and also decreased with an increase in m_h. Therefore, the decrease in W_{lv} could have been caused by the decrease in heart frequency (cf. 4.4). However, we found that under certain experimental conditions also at constant heart rate the maximum value of W_{lv} (W_{lv} (max)) per unit of dry heart mass decreases when the mass of the heart increases. This is caused by the existence of a relationship between m_h and W_{lv} (max), which cannot be described by a straight line through the origin. We also found that this relationship depends on the hydrostatic aortic pressure (P_{aoh}). Two hypothetical relationships are shown in Fig. 3.1.

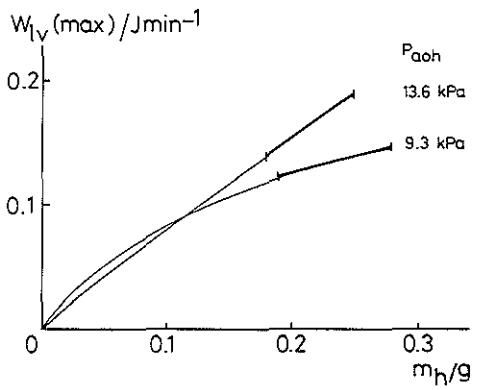

Fig. 3.1 Hypothetical relationships between dry heart mass (m_h) and maximal external left ventricular work (W_{lv}(max)) at 2 values of the hydrostatic aortic pressure (P_{aoh}).
The courses of the relationships are based on the regression lines of the relationship found between m_h and W_{lv}(max) (thick lineparts; cf. Fig. 3.2 A and D) and the origin.

TABLE 3.1 Mean values (± SEM) of dry heart mass (m_h) and maximal external left ventricular work ($W_{lv}(max)$) of hearts of rats fed various dietary fats for 4 to 6 weeks.

Experiment	Dietary group	LA/SFA	m_h / mg	$W_{lv}(max)$ / J min^{-1}		b	b'
				determined	adjusted[1])		
1, n=10	2 SSO + 43 L	0.3	221 ± 6	1.25 ± 0.03	1.25 ± 0.03	1.21	2.40 ± 1.13
	45 SSO	5.7	221 ± 6	1.35 ± 0.04	1.35 ± 0.03	3.72	P<0.05
2, n=10	2 SSO + 33 L	0.3	220 ± 4	1.30 ± 0.04	1.30 ± 0.04	1.75	
	13 SSO + 22 L	1.0	219 ± 3	1.27 ± 0.06	1.27 ± 0.05	9.64	5.26 ± 1.73
	24 SSO + 11 L	2.2	219 ± 3	1.40 ± 0.04	1.40 ± 0.05	2.25	P<0.01
	35 SSO	5.7	217 ± 3	1.38 ± 0.03	1.40 ± 0.04	3.73	
3, n=7	2 SSO + 33 L	0.3	212 ± 7	1.32 ± 0.05	1.34 ± 0.04	3.37	2.47 ± 1.55
	35 SSO	5.5	218 ± 7	1.46 ± 0.05	1.46 ± 0.04	1.89	P>0.1
4, n=9	35 L	0.2	238 ± 5	1.32 ± 0.05	1.27 ± 0.04	2.55	
	17.5 SSO + 17.5 L	1.3	233 ± 7	1.35 ± 0.05	1.32 ± 0.04	−2.56	1.23 ± 1.21
	35 SSO	5.5	233 ± 7	1.42 ± 0.06	1.39 ± 0.04	1.70	P>0.1
5, n=11	10 SSO + 25 L	0.8	213 ± 4	1.21 ± 0.03	1.23 ± 0.03	0.99	
	15 SSO + 20 L	1.1	214 ± 8	1.22 ± 0.03	1.24 ± 0.03	4.85	3.53 ± 0.13
	20 SSO + 15 L	1.7	213 ± 5	1.25 ± 0.04	1.28 ± 0.03	1.44	P<0.001
	25 SSO + 10 L	2.5	226 ± 5	1.31 ± 0.03	1.29 ± 0.03	3.80	
6, n=10	6 SSO + 29 L	0.50	214 ± 6	1.61 ± 0.04	1.65 ± 0.05	8.00	
	21 SSO + 14 L	1.75	217 ± 4	1.62 ± 0.05	1.64 ± 0.04	12.56	7.50 ± 1.41
	28 SSO + 7 L	3.00	218 ± 3	1.69 ± 0.04	1.71 ± 0.04	−1.02	P<0.001
	32 SSO + 3 L	4.25	221 ± 4	1.71 ± 0.04	1.70 ± 0.04	7.34	
	35 SSO	5.50	207 ± 4	1.64 ± 0.03	1.75 ± 0.04	7.18	

2 SSO: 2 en% sunflowerseed oil; 43 L: 43 en% lard; LA/SFA: dietary ratio of linoleic acid and saturated fatty acids.

[1]) Adjusted to m_h = 0.220 g, by analysis of covariance.

b: regression coefficient of the relationship between m_h and the adjusted values of $W_{lv}(max)$; b': pooled regression coefficient. In the calculation of b and b' m_h was expressed per g.

In all experiments the experimental circumstances were similar, except in experiment 6 where the hydrostatic aortic pressure (P_{aoh}) was 13.6 kPa, whereas in the other experiments P_{aoh} was 9.3 kPa. In all experiments the stimulation frequency was 360 min^{-1}.

TABLE 3.2 Effect of hydrostatic aortic pressure (P_{aoh}) on the regression coefficients of the relationships between dry heart mass (m_h) and both maximal external left ventricular work (W_{lv}(max)), cardiac output ($\dot{Q}_{lv}$) and peak aortic pressure (P_{aop}) in the working rat heart.

Experiment	n		P_{aoh}	m_h versus W_{lv}(max)		m_h versus $\dot{Q}_{lv}$		m_h versus P_{aop}	
	Groups	Hearts	/kPa	b' [1]	P [2]	b' [1]	P [2]	b' [1]	P [2]
1 - 5	15	141	9.3	2.89 ± 0.57 [3]	2×10^{-6}	201.5 ± 34.5 [4]	$<1 \times 10^{-6}$	4.2 ± 5.05 [5]	0.41
6	5	51	13.6	7.50 ± 1.41 [3]	3×10^{-6}	352.5 ± 65.8 [4]	3×10^{-6}	35.4 ± 12.2 [5]	0.006

1) Pooled regression coefficient ± SEM.
2) Probability.
3) $P<0.01$.
4) $P=0.03$.
5) $P<0.01$.

The statistical analysis of the 3 relationships was performed by analysis of covariance. Pooled regression coefficients were calculated, because analysis of covariance did not show any systematic difference in the regression coefficients within the groups of the experiments 1-5 and experiment 6.

3.2 RELATIONSHIP BETWEEN DRY HEART MASS AND MAXIMAL EXTERNAL LEFT VENTRICULAR WORK

Data of 6 experiments in which rats had been fed diets containing sunflower seed oil (SSO), lard (L) or mixtures of SSO and L for 4 to 6 weeks have been used to evaluate the relationship between m_h and $W_{lv}(max)$. The dietary groups in the experiments with the corresponding mean values of m_h and $W_{lv}(max)$ are listed in Table 3.1. The relationships between m_h and $W_{lv}(max)$ in the various experiments were statistically evaluated by analysis of covariance. For the m_h values investigated, this analysis shows that $W_{lv}(max)$ is linearly related to m_h. Moreover, for the dietary groups within each experiment, the regression coefficients of the relationship between m_h and W_{lv} did not differ systematically ($P \geqslant 0.23$) showing that the type of dietary fat used did not affect them. Therefore, the pooled regression coefficient for each experiment was calculated (b', Table 3.1). Analysis of covariance further showed that the regression coefficients (b in Table 3.1) within the dietary groups of the experiments 1-5 did not mutually differ systematically ($P = 0.21$), but within the dietary groups of the experiments 1-6 they did ($P = 0.023$). Therefore, the pooled regression coefficient for the experiments 1-5 was calculated. This regression coefficient differs systematically from the pooled regression coefficient for experiment 6 (Table 3.2). As the difference between the experiments 1-5 and experiment 6 is the difference in P_{aoh} it can be concluded that P_{aoh} influences the relationship between m_h and $W_{lv}(max)$: at $P_{aoh} = 9.3$ kPa this relationship is curvilinear, whereas at $P_{aoh} = 13.6$ kPa it is almost linear (Fig. 3.1). Thus it would seem that bigger hearts possess a capacity to perform external work which is not used at $P_{aoh} = 9.3$ kPa, but which is at $P_{aoh} = 13.6$ kPa.

For all the hearts of the experiments 1-5 and experiment 6 $W_{lv}(max)$ has been plotted against m_h in Fig. 3.2 A and D.

3.3 RELATIONSHIP BETWEEN DRY HEART MASS AND BOTH PEAK AORTIC PRESSURE AND CARDIAC OUTPUT

As W_{lv} is calculated from peak aortic pressure (P_{aop}) and left ventricular output ($\dot{Q}_{lv}$) (cf. 2.5), it was investigated to what extent P_{aop} and $\dot{Q}_{lv}$ (corresponding to $W_{lv}(max)$) depended on m_h. For all the groups of the experiments 1-5 ($P_{aoh} = 9.3$ kPa) and experiment 6 ($P_{aoh} = 13.6$ kPa) the relationship between m_h and both P_{aop} and $\dot{Q}_{lv}$ were evaluated by analysis of covariance. This analysis shows for the experiments 1-5 a systematic relationship between m_h and $\dot{Q}_{lv}$, but no relationship between m_h and P_{aop} (Table 3.2; Fig. 3.2.); however, in experiment 6 m_h is related both to $\dot{Q}_{lv}$ and P_{aop}. The difference between the experiments 1-5 and experiment 6 is the differen-

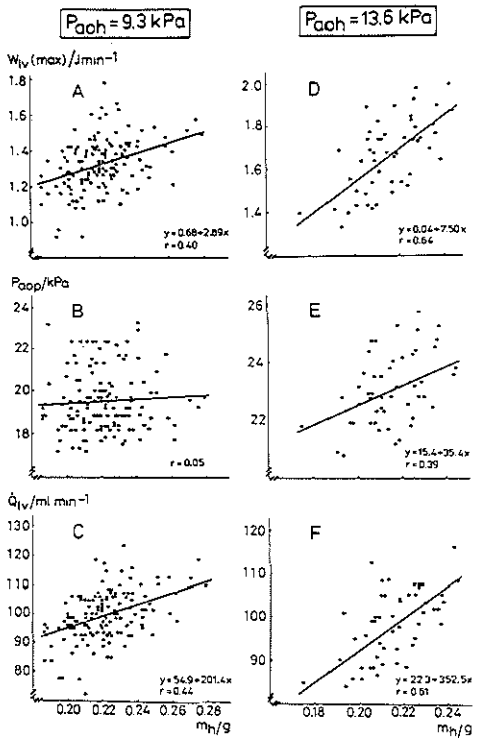

Fig. 3.2 Relationships between dry heart mass (m_h) and both maximal external left ventricular work (W_{lv}(max)), peak aortic pressure (P_{aop}) and cardiac output ($\dot{Q}_{lv}$) at 2 values of the hydrostatic aortic pressure (P_{aoh}) in the working rat heart.
The results of experiments 1-5 are shown in the panels A, B and C, those of experiment 6 in the panels D, E and F. More than 1 point can be present on the same location. For details of the experiments see Table 3.1. Note the interruptions in the axes and the differences in the scales between the left and right parts of the figure. For statistical analysis see Table 3.2.

ce in P_{aoh}. Therefore, it can be concluded that P_{aoh} influences the relationship between m_h and P_{aop}, and as P_{aop} is representative of the maximum value of the left ventricular systolic pressure (P_{lvs}) (cf. Fig. 2.7), also the relationship between m_h and P_{lvs}.

3.4 CONSEQUENCES OF EXPRESSING EXTERNAL LEFT VENTRICULAR WORK PER UNIT OF DRY HEART MASS

In this section the consequences of expressing W_{lv} per g of dry heart mass (W_{lv} / m_h) will be evaluated. Dry heart mass and the type of dietary fat are two

variables which affect W_{lv}. In this study, the effect of the type of dietary fat on W_{lv} has been investigated. Therefore, m_h is another variable for which should be corrected using the analysis of covariance, which is a well known statistical technique and therefore the evaluation can be short.
A hypothetical experiment consists of 4 groups. The mean values of $W_{lv}(max)$ of the 4 groups are represented by the symbols D, E, F and G (Fig. 3.3). It is assumed that analysis of covariance did not show systematic differences between the regression coefficients of the relationship between m_h and $W_{lv}(max)$ of the 4 groups. These relationships which were calculated using the pooled regression coefficient are represented in Fig. 3.3 by the regression lines through the points D, E, F and G. The length of the lines represents the variation in m_h. From the figure it is clear that the $W_{lv}(max)$ values D and G are different, and that the $W_{lv}(max)$ values F and E, when they are adjusted to the value of m_h corresponding to the $W_{lv}(max)$ values D and G, will be equal, but different from the $W_{lv}(max)$ values D and G. However, when the $W_{lv}(max)$ values D, E, F and G are divided by the corresponding values of m_h, then F/m_h and D/m_h will be equal, as well as G/m_h and E/m_h, but F/m_h and D/m_h will be different from G/m_h and E/m_h.

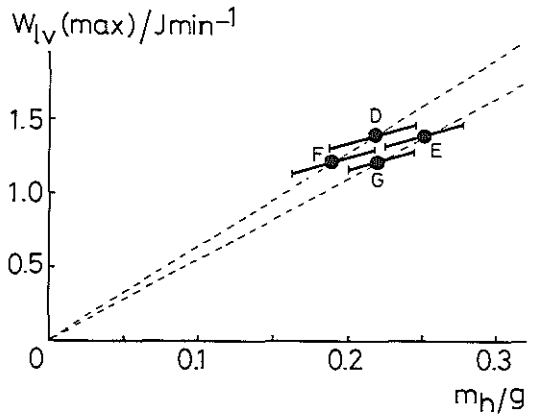

Fig. 3.3 Relationship between dry heart mass (m_h) and maximal external left ventricular work ($W_{lv}(max)$) in 4 groups of hearts. For explanation see section 3.4.

This exposition shows that using $W_{lv}(max)$ divided by m_h can lead to a wrong interpretation of the results. Only when the mean values of m_h are about the same as in the case of D and G, the expression of $W_{lv}(max)$ per unit of mass will not affect the relative difference between the mean values of $W_{lv}(max)$. This was the case in the present study. Feeding the various dietary fats never led to appreciable differences in m_h of the groups (see Table 3.1). In

accordance with most of the literature [1-5] W_{lv}/m_h was used as quantity in the Addenda. However, in the following chapters external left ventricular work is used as such.

3.5 RELATIONSHIP BETWEEN DRY HEART MASS AND CORONARY FLOW RATE

In the Addenda, coronary flow rate ($\dot{Q}_{cor}$) is also expressed per g of dry heart mass. It is reasonable to assume that a big heart contains more coronary vessels than a small heart and consequently will show a higher $\dot{Q}_{cor}$. However, this latter is only true to some extent. In the Langendorff-perfused guinea pig (Addendum VII) and rabbit heart (Addendum II), $\dot{Q}_{cor}$ when expressed in ml min^{-1}, is only little higher than in the rat heart, whereas their heart masses are 3-6 times that of the rat heart. Consequently, when $\dot{Q}_{cor}$ is expressed per g of dry heart mass this results in rather low values of $\dot{Q}_{cor}$ for the guinea-pig and rabbit hearts.

To illustrate in the Langendorff-perfused rat heart the effect of heart mass on $\dot{Q}_{cor}$, the values of $\dot{Q}_{cor}$ obtained in two experiments are plotted against m_h

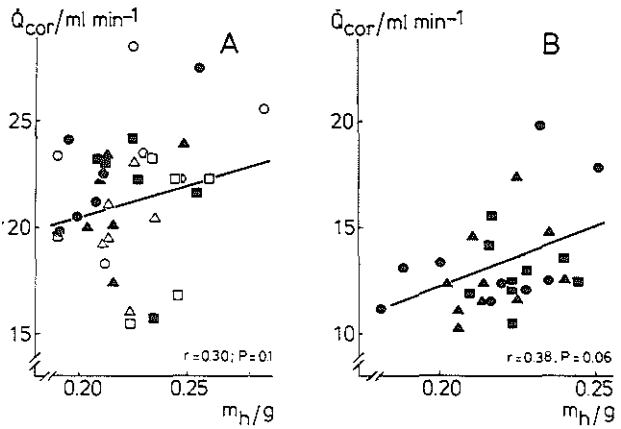

Fig. 3.4 Relationship between dry heart mass (m_h) and coronary flow rate ($\dot{Q}_{cor}$) in the Langendorff-perfused rat heart.

A. Perfusion pressure 10.7 kPa. Six groups of rats were fed diets containing various fats for 1 week.

●, SSO; ○, SBO; ■, OO; △, PO; □, L; ▲, $HSBO_2$. For further details of the experiment see Table 4.2.

B. Perfusion pressure 9.3 kPa. $\dot{Q}_{cor}$ was determined at t = 11 min. The relationship between m_h and $\dot{Q}_{cor}$ was also determined at t = 3 and 21 min, but as the relationships were similar, only that for $\dot{Q}_{cor}$ at t = 11 min is shown.

●, SSO; ■, L; ▲, HCO. For further details see Fig. 5.2.

(Fig. 3.4). Although Fig. 3.4 indicates that the relationship between m_h and $\dot{Q}_{cor}$ is similar to the relationship between m_h and $W_{lv}(max)$, in the range of the determined values of m_h there was no systematic relationship between m_h and $\dot{Q}_{cor}$. Therefore, in the next chapters the values of $\dot{Q}_{cor}$ obtained in the Langendorff-perfused heart will be expressed in ml min^{-1} and the mean m_h values of the groups will be given together with the data on $\dot{Q}_{cor}$.
In the working heart $\dot{Q}_{cor}$ is related to W_{lv} (Addendum III, Figs. 1 and 2). Because W_{lv} is related to m_h this implies that also $\dot{Q}_{cor}$ will be related to m_h in a similar way as W_{lv}. Therefore, also the values of $\dot{Q}_{cor}$ obtained in the working heart will be expressed in ml min^{-1}.

3.6 SUMMARY

In the working rat heart, heart mass is positively related to maximal external left ventricular work ($W_{lv}(max)$). In the present study dry heart mass (m_h) has been taken as index of heart mass. It was found that the relationship between m_h and $W_{lv}(max)$ depends on the hydrostatic aortic pressure (P_{aoh}) : at P_{aoh} = 9.3 kPa the relationship is curvilinear, whereas at P_{aoh} = 13.6 kPa the relationship can be considered to be linear.
Feeding sunflowerseed oil, lard or mixtures of these fats did not lead to systematic differences in the regression coefficients of the relationships between m_h and $W_{lv}(max)$.
In the Langendorff-perfused heart, coronary flow rate ($\dot{Q}_{cor}$) showed a similar relationship as between m_h and $W_{lv}(max)$ at P_{aoh} = 9.3 kPa. In the working heart $\dot{Q}_{cor}$ will be related to m_h, because $\dot{Q}_{cor}$ is related to W_{lv}.

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4 EFFECTS OF DIETARY FATS IN THE ISOLATED RAT HEART

4.1 SUNFLOWERSEED OIL VERSUS OTHER TYPES OF DIETARY FATS

4.1.1 Introduction

The investigation of the effects of feeding to rats diets containing sunflowerseed oil (SSO), hydrogenated coconut oil (HCO) and lard (L) on coronary flow rate ($\dot{Q}_{cor}$), myocardial oxygen consumption ($\dot{V}^{m}_{O_2}$) and other metabolic aspects in the Langendorff-perfused heart, and in the working heart also on spontaneous frequency (f_s) and external left ventricular work (W_{lv}) have been described in Addenda III and IV. In this section the main results of these investigations will be presented together with some additional data. Besides, results obtained after feeding other dietary fats will also be presented.

4.1.2 Effects on body mass, dry heart mass and spontaneous heart frequency

In Addendum IV (Table III) it is shown that feeding SSO or mixtures of SSO and L for 4 weeks does not lead to differences in body mass (m_b) and dry heart mass (m_h). Also feeding other dietary fats did not result in systematic differences in m_b and m_h between the dietary groups (Table 4.1).

In most experiments f_s was also determined. As f_s usually declines during perfusion, f_s was measured before electrical pacing of the heart started, at about 10 min after the start of perfusion. Feeding the various dietary fats did not lead to systematic differences in f_s between the dietary groups (Addendum IV, Table IV; Table 4.1). However, in all experiments in which L was compared with SSO, f_s of the L groups was lower than f_s of the SSO groups. In one experiment, an extra amount of vitamin E was added to a diet with 33 en% L. It was found that f_s of this group was higher than that of the group without this extra amount of vitamin E (Addendum IV, Table IV). This effect has not been investigated further. Nevertheless, the lower f_s of the lard groups is most probably not caused by the lower amount of vitamin E in the diets which contain L, as the other dietary fats like HCO which also contains

a very small amount of vitamin E [3] did not decrease f_s in comparison with the SSO groups (Table 4.1).

TABLE 4.1 Body mass (m_b) at the start and end of the experimental feeding period, dry heart mass (m_h) and spontaneous frequency (f_s) in the isolated heart of rats fed various dietary fats.

Experiment	Dietary group	Feeding time /weeks	m_b/g		m_h/mg	f_s/min^{-1}
			start	end		
a	50 SSO	3	158 ± 3	245 ± 5	180 ± 6	n.d.
	45 HCO + 5 SSO		157 ± 2	250 ± 5	182 ± 4	n.d.
b	30 SSO	6	232 ± 4	340 ± 7	218 ± 5	321 ± 8
	28 L + 2 SSO		232 ± 4	346 ± 8	218 ± 4	303 ± 10
	45 SSO		230 ± 3	348 ± 9	221 ± 6	315 ± 7
	43 L + 2 SSO		232 ± 4	369 ± 8	221 ± 6	300 ± 7
c	35 SSO	4	330 ± 8	398 ± 11	254 ± 8	317 ± 9
	35 L		330 ± 6	385 ± 8	243 ± 3	294 ± 9
	30 HSBO + 5 SSO		327 ± 7	385 ± 9	244 ± 4	320 ± 12
d	35 SSO	4	222 ± 4	325 ± 10	221 ± 6	340 ± 11
	30 HCO + 5 SSO		223 ± 4	325 ± 10	221 ± 6	354 ± 7
	30 HSBO + 5 SSO		222 ± 4	332 ± 6	220 ± 5	331 ± 12
e	35 SSO	6	229 ± 4	362 ± 10	233 ± 7	330 ± 8
	35 SBO		230 ± 4	354 ± 5	236 ± 6	334 ± 12
	35 OO		230 ± 4	370 ± 6	234 ± 5	311 ± 7
	35 LSO		230 ± 4	354 ± 8	226 ± 4	324 ± 5
	35 PO		229 ± 3	370 ± 11	226 ± 8	321 ± 14
	35 L		230 ± 3	365 ± 9	238 ± 5	304 ± 5
	17.5 SSO + 17.5 L		230 ± 5	369 ± 10	233 ± 7	329 ± 9
	30 HSBO + 5 SSO		231 ± 4	360 ± 6	223 ± 4	309 ± 7

Mean values ± SEM, n = 9-10; n.d. = not determined. 50 SSO: 50 en% sunflowerseed oil, etc. (cf. Table 2.1).
Spontaneous heart frequency was determined in the Langendorff-perfused heart (experiment c) and in the working heart at left ventricular filling pressure = 0.80 kPa (experiments b, d, and e). Experiments a and b have been described in Addenda III (Fig. 2) and IV (Fig. 1 C), respectively. For further data on the experiments d and e see Table 4.3.

4.1.3 Effect on coronary flow rate in the Langendorff-perfused heart

When rats were fed saturated fats like HCO or L for about 1 or 6 weeks $\dot{Q}_{cor}$ was found to be lower than $\dot{Q}_{cor}$ of the hearts of rats fed SSO (Addenda III (Table IV) and IV (Results section)). When diets containing mixtures of SSO and HCO were fed to rats for 3 days $\dot{Q}_{cor}$ was positively related to the amount of SSO (Fig. 4.1).
Also other dietary fats may influence $\dot{Q}_{cor}$ as shown in Table 4.2. Due to the

great number of groups in these experiments - as a result of which statistical discrimination diminishes - the differences in $\dot{Q}_{cor}$ are not systematic. This table shows again that feeding SSO, in comparison with saturated fats like L and palm oil (PO), increased $\dot{Q}_{cor}$. $\dot{Q}_{cor}$ of the olive oil (OO) group is similar to $\dot{Q}_{cor}$ of the saturated fat groups. Therefore, it might be concluded that the linoleic acid from SSO and also from soyabean oil (SO) increased $\dot{Q}_{cor}$. $\dot{Q}_{cor}$ of the hydrogenated soyabean oil (HSBO) groups lies between those of the SSO and the saturated fat groups, which might indicate that also other dietary fatty acids can increase $\dot{Q}_{cor}$.

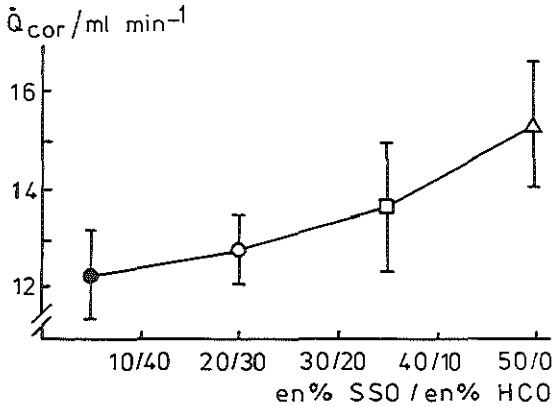

Fig. 4.1 Relationship between the dietary ratio of sunflowerseed oil (SSO) and hydrogenated coconut oil (HCO) and coronary flow rate ($\dot{Q}_{cor}$) in the Langendorff-perfused heart.
Four groups of rats were fed diets containing mixtures of SSO and HCO for 3 days. Analysis of variance showed a significant relationship ($P > 0.05$) between the amount of dietary SSO and $\dot{Q}_{cor}$. Perfusion pressure 9.3 kPa; mean values of $\dot{Q}_{cor} \pm$ SEM, $n = 9$. Mean values of dry heart mass ranged from 184 to 188 mg.

As already discussed (cf. 2.4) $\dot{Q}_{cor}$ in the Langendorff-perfused heart is rather high compared to coronary blood flow in the intact animal. However, the values of $\dot{Q}_{cor}$ obtained under normoxic conditions are still much lower than the maximal values obtained by perfusion with vasodilators like adenosine (Addendum III, Table IV) and prostaglandins (Addendum VI); by these agents $\dot{Q}_{cor}$ was doubled. As a result of maximal vasodilation by perfusion with adenosine the differences in $\dot{Q}_{cor}$ of the dietary groups disappeared (Addendum III, Table IV; Fig. 4.2). Therefore, it may be concluded that the differences in $\dot{Q}_{cor}$ between the dietary groups were caused by influences on the regulation of $\dot{Q}_{cor}$ in the Langendorff-perfused heart.

TABLE 4.2 Dry heart mass (m_h) and coronary flow rate ($\dot{Q}_{cor}$) in the Langendorff-perfused heart of rats fed various dietary fats for 1 week.

Dietary group	LA / SFA[1])	Experiment 1		Experiment 2	
		m_h/mg	$\dot{Q}_{cor}$ / ml min^{-1}	m_h/mg	$\dot{Q}_{cor}$ / ml min^{-1}
50 SSO	5.5	167 ± 4	19.4 ± 0.5	227 ± 7	21.6 ± 1.2
50 SBO	3.4	162 ± 4	18.0 ± 0.8	221 ± 4	22.8 ± 1.6
50 OO	0.6	161 ± 5	14.4 ± 1.0[2])	234 ± 10	20.6 ± 1.2
50 PO	0.2	159 ± 2	16.4 ± 0.6	218 ± 6	18.8 ± 1.0
50 L	0.2	156 ± 6	15.7 ± 1.3	231 ± 14	19.0 ± 1.2
50 HSBO	0.0	156 ± 5	17.2 ± 1.3	210 ± 10	20.2 ± 1.0

Mean values ± SEM; n = 6. Perfusion pressure = 10.7 kPa. 50 SSO: 50 en% sunflowerseed oil, etc. (cf. Table 2.1).

[1]) LA / SFA: ratio of dietary linoleic acid and saturated fatty acids.

[2]) Newman Keuls multiple range test showed that only $\dot{Q}_{cor}$ of the 50 OO group in experiment 1 differs systematically ($P<0.05$) from $\dot{Q}_{cor}$ of the 50 SSO group.

TABLE 4.3 Maximal external left ventricular work (W_{lv}(max)) and coronary flow rate ($\dot{Q}_{cor}$) in the working heart of rats fed various dietary fats.

Dietary group	LA/SFA[1])	Feeding time /weeks	W_{lv}(max) / J min^{-1} determined	W_{lv}(max) / J min^{-1} adjusted[2])	$\dot{Q}_{cor}$ / ml min^{-1}
35 SSO	5.5	4	1.27 ± 0.05	1.27 ± 0.04	24.5 ± 0.7
30 HCO + 5 SSO	0.5		1.14 ± 0.04	1.14 ± 0.04	22.3 ± 0.2
30 HSBO + 5 SSO	0.5		1.17 ± 0.02	1.17 ± 0.04	23.5 ± 0.4
35 SSO	5.5	6	1.44 ± 0.06	1.40 ± 0.04	28.2 ± 0.7
35 SBO	3.4		1.32 ± 0.05	1.30 ± 0.04	28.8 ± 0.7
35 OO	0.6		1.31 ± 0.03	1.28 ± 0.04	28.1 ± 0.7
35 LSO	0.5		1.28 ± 0.05	1.27 ± 0.04	26.2 ± 1.1
35 PO	0.2		1.28 ± 0.05	1.27 ± 0.04	26.7 ± 0.9
35 L	0.2		1.33 ± 0.05	1.30 ± 0.04	28.1 ± 1.0
17.5 SSO + 17.5 L	1.3		1.36 ± 0.05	1.34 ± 0.04	28.2 ± 1.2
30 HSBO + 5 SSO	0.5		1.36 ± 0.03	1.35 ± 0.04	28.1 ± 0.9

Mean values ± SEM; n = 9. 35 SSO: 35 en% sunflowerseed oil, etc. (cf. Table 2.1).

[1]) LA / SFA: ratio of dietary linoleic acid and saturated fatty acids.

[2]) Adjusted values of W_{lv}(max) calculated for m_h = 220 mg by analysis of covariance (cf. chapter 3).

For mean dry heart mass see Table 4.1, experiments d and e.

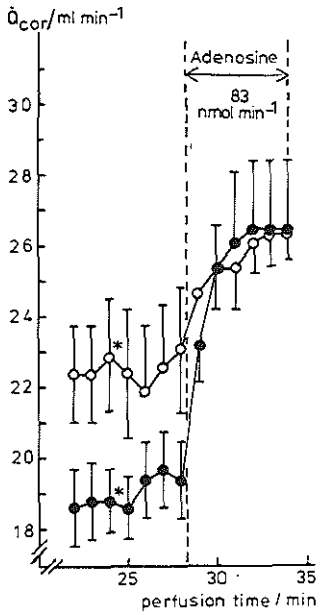

Fig. 4.2 Effect of adenosine on coronary flow rate ($\dot{Q}_{cor}$) in the Langendorff-perfused heart of rats fed 50 en% soyabean oil (o————o) or palm oil (●————●) for 1 week.
At perfusion time 28 min adenosine was added to the perfusion fluid. Perfusion pressure 10.7 kPa; mean values ± SEM; n = 6; *$P_2 < 0.05$. For mean dry heart mass see Table 4.2.

4.1.4 Effect on coronary flow rate in the working heart

In the working heart, $\dot{Q}_{cor}$ increased linearly with W_{lv} (Addendum III, Figs. 1 and 2), and is higher than in the Langendorff-perfused heart. At the maximal values of W_{lv}(W_{lv}(max)), very high values of $\dot{Q}_{cor}$ were obtained. However, even at W_{lv}(max), $\dot{Q}_{cor}$ was not yet maximal as $\dot{Q}_{cor}$ could still be increased by perfusion with prostaglandins (Addendum VI, Results section) or epinephrine (cf. Fig. 2.10). In the latter case, the increase in $\dot{Q}_{cor}$ will not only have been caused by the coronary vasodilation, but also by the increase in the mean aortic pressure ($\overline{P}_{ao}$) due to the increase in the left ventricular contractile force.

In the working heart, feeding SSO in comparison with other dietary fats did not lead to systematic differences in $\dot{Q}_{cor}$ (Addenda III (Figs. 1 and 2) and IV (Table V); Table 4.3). It is probable that differences in $\dot{Q}_{cor}$ found in the Langendorff-perfused heart are not found in the working heart, because of the regulatory influence of cardiac work on $\dot{Q}_{cor}$. As a result of this, like in the

case of the perfusion with adenosine, influences of the dietary fats on $\dot{Q}_{cor}$ were not observed.

4.1.5 Effect on external left ventricular work

In Addenda III and IV it is shown that dietary fats affect the relationship between left ventricular filling pressure (P_{lvf}) and W_{lv}; the function curves of the SSO groups, in comparison with those of the HCO and L groups, have been shifted upward. Addendum IV also shows that the value of P_{lvf} at which $W_{lv}(max)$ is obtained was the same for the dietary fat groups. If this would not have been the case, an effect on the compliance of the heart would have been probable. Therefore, the effect of dietary fat on the function curves can be considered to be an effect on the contractility of the heart (cf. 2.5).

Since in all experiments it was found that the type of dietary fat did not affect the value of P_{lvf} at which $W_{lv}(max)$ was obtained, generally only $W_{lv}(max)$ is given and not the upper part of the function curve (which was always determined). For a good understanding it should be noted again that $W_{lv}(max)$ is the peak value of the function curve. $W_{lv}(max)$ can increase as a result of higher values of the hydrostatic aortic pressure (P_{aoh}) (cf. Fig. 4.5 A) or by perfusion with catecholamines (cf. Fig. 2.10).

In two other experiments a number of dietary fats were fed for 4 and 6 weeks, and $W_{lv}(max)$ was determined. The $W_{lv}(max)$ values, listed in Table 4.3, confirm the result mentioned above that feeding SSO, in comparison with other dietary fats, increases $W_{lv}(max)$.

The $W_{lv}(max)$ values of the groups fed HSBO are also somewhat higher than those of the other groups (except the SSO groups) which might indicate that also feeding HSBO, in comparison with the other dietary fats used, increased $W_{lv}(max)$. Whether this is significant has to be investigated further, but the finding is in accordance with the intermediate position of $\dot{Q}_{cor}$ of the HSBO groups in the Langendorff-perfused heart (Table 4.2).

In the experiments described in Addendum III the values of W_{lv} are lower than in most other experiments. This was caused by the use of an aortic cannula with a smaller inner diameter (0.12 cm). Therefore, the effect on $W_{lv}(max)$ of feeding HCO compared to SSO was investigated again in an experiment in which an aortic cannula with an inner diameter of 0.21 cm was used. The results summarized in Table 4.3 show that the relative difference in the $W_{lv}(max)$ values between the HCO and SSO groups was not affected by the inner diameter of the aortic cannula.

4.1.6 Effect on myocardial oxygen consumption

The effect of feeding SSO, HCO and L on $\dot{V}^{m}_{O_2}$ has been studied both in the Langendorff-perfused and working heart. Neither in the Langendorff-perfus-

ed heart (Addendum III, Table V), nor in the working heart (Addendum IV, Table V) did feeding these dietary fats result in systematic differences in $\dot{V}^m_{O_2}$. $\dot{V}^m_{O_2}$ is related to the amount of cardiac work. Therefore, the relationship between W_{lv} and $\dot{V}^m_{O_2}$ was determined after feeding SSO and HCO. Addendum III (Figs. 1 and 2) shows that feeding these fats did not lead to any difference in this relationship.

4.1.7 Effects on the respiratory properties of isolated heart mitochondria

The respiratory properties of isolated mitochondria were determined after feeding SSO and L for 6 weeks. Addendum IV (Table VI) shows that feeding these dietary fats resulted neither in differences in the rate of oxygen consumption, respiratory control index, nor in the ADP/O ratio. Therefore, the rate of ATP synthesis in the isolated heart mitochondria was not affected.

4.1.8 Effects of experimental feeding time on coronary flow rate and external left ventricular work

As already mentioned (4.1.3) feeding SSO, in comparison with HCO or L, for about 1 week increased $\dot{Q}_{cor}$ in the Langendorff-perfused heart. Feeding SSO and HCO for only 4 days also resulted in a difference in W_{lv} between the two groups (Addendum III, Fig. 1). However, feeding SSO in comparison with L for 1 week did not lead to systematic differences in W_{lv}(max) (Addendum IV, Fig. 1 A).

Also in another experiment in which groups of rats were fed diets containing 35en% of OO, PO, L, HSBO or SSO for 1 week, no differences were found in W_{lv}(max) (0.75 ± 0.04, 0.79 ± 0.03, 0.74 ± 0.04, 0.76 ± 0.04, 0.79 ± 0.03 J min^{-1}, respectively; inner diameter aortic cannula 0.12 cm). Apparently, feeding of various dietary fats for 1 week, except HCO, does not lead to differences in W_{lv}(max).

4.1.9 Effect of the amount of dietary fat on external left ventricular work

The effect of the amount of dietary fat on W_{lv}(max) was studied only to a limited extent. The results shown in Addendum IV (Figs. 1A and 1C) might indicate an influence of the total amount of dietary fat on W_{lv}(max). Also in another experiment in which 5 groups of rats were fed diets containing increasing amounts of SSO for about 1 week, only a weak indication was found of an effect of the amount of fat on W_{lv}(max) (Table 4.4). In the latter experiment the relationships between W_{lv} and both $\dot{Q}_{cor}$ and $\dot{V}^m_{O_2}$ as found in Addendum III were confirmed (not shown).

4.1.10 Effect on the fatty acid composition of myocardial phospholipds

Feeding SSO or HCO for 5 days did not result in appreciable differences in the fatty acid composition of the myocardial phospholipids (Addendum III,

TABLE 4.4 Influences of diets containing increasing amounts of sunflowerseed oil (SSO) fed to rats for 1 week on maximal external left ventricular work ($W_{lv}(max)$) and coronary flow rate ($\dot{Q}_{cor}$) in the working heart.

Dietary group	m_h/mg	$W_{lv}(max)$/J min^{-1}		$\dot{Q}_{cor}$/ml min^{-1}
		determined	adjusted[1])	
5 SSO	175 ± 4	0.67 ± 0.03	0.68 ± 0.03	18.4 ± 0.5
15 SSO	180 ± 7	0.68 ± 0.02	0.68 ± 0.03	18.2 ± 0.4
25 SSO	174 ± 5	0.73 ± 0.04	0.76 ± 0.03	19.7 ± 0.5
35 SSO	188 ± 3	0.82 ± 0.05	0.77 ± 0.03	20.9 ± 1.1
45 SSO	183 ± 6	0.71 ± 0.02	0.70 ± 0.03	19.9 ± 0.4

Mean values ± SEM; n = 9. 5 SSO: 5 en% sunflowerseed oil, etc. In the diets starch was exchanged for SSO.

The $W_{lv}(max)$ values are lower than generally found, because an aortic cannula with an inner diameter of 0.12 cm was used instead of 0.21 cm.

[1]) Adjusted to m_h = 178 mg (mean value) by analysis of covariance. The differences in the adjusted values of $W_{lv}(max)$ are not systematic (P = 0.18).

TABLE 4.5 Fatty acid composition (%; w / w) of total phospholipids of left ventricular tissue of rats fed mixtures (total 35 en%) of sunflowerseed oil (SSO) and lard (L) for 6 weeks.

Fatty acid	Dietary group			
	10 SSO + 25 L	15 SSO + 20 L	20 SSO + 15 L	25 SSO + 10 L
C 14:0	0.3	0.1	0.3	—
C 16:0	9.9	9.5	9.0	8.3
C 16:1 (n-7)	0.5	0.3	0.4	0.4
C 18:0	18.9	20.3	15.9	18.2
C 18:1 (n-9)	6.2	6.3	5.1	5.6
C 18:2 (n-6)	17.9	19.0	21.4	22.6
C 18:3 (n-3)	0.5	0.3	0.3	0.4
C 20:3 (n-6)	0.3	0.2	0.3	0.4
C 20:4 (n-6)	24.2	23.7	27.1	26.0
C 20:5 (n-3)	1.9	1.1	0.9	0.5
C 22:4 (n-6)	1.5	1.7	1.9	2.5
C 22:5 (n-6)	2.5	4.2	4.5	4.1
C 22:5 (n-3)	1.9	1.4	1.2	1.2
C 22:6 (n-3)	9.9	9.4	9.1	7.2

The fatty acid composition of the phospholipids was determined in pooled samples of 10 hearts (cf. 2.2.4). The hearts were taken after perfusion (section 4.3.2, experiment 5).

Table VI). The influence of dietary fats on the fatty acid composition of the phospholipids is, however, very clear after 4 to 6 weeks of feeding (Addendum IV (Table VII); Tables 4.5 and 6.5).When groups of rats were fed diets containing mixtures of SSO and L, the relative amounts of linoleic acid and linoleic acid-derived fatty acids in the phospholipids increased with the dietary amount of linoleic acid (C20:4(n-6) only to a small extent; Table 4.5; Fig. 4.3). Generally, by feeding SSO-rich diets in comparison with L-rich diets, mainly C18:2(n-6), C22:4(n-6) and C22:5(n-6) increased, and C18:1(n-9), C22:5(n-3) and C22:6(n-3) decreased; the amounts of C18:0 and C20:4(n-6) were hardly affected by the dietary regimens used.

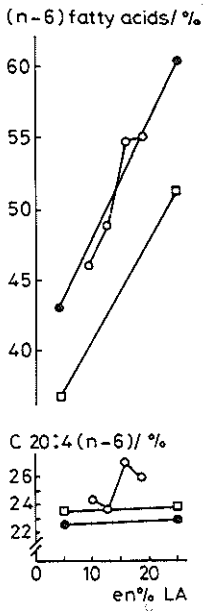

Fig. 4.3 Relationship between the amount of dietary linoleic acid (LA) and the total amount (%, w / w) of the (n-6) fatty acids C 18:2, C 20:4, C 22:4 and C 22:5 and the amount of arachidonic acid (C 20:4(n-6)), respectively in phospholipids of rat heart.
o———o, mitochondria. Diets were fed for 6 weeks (Addendum IV, Table VII).
●———●, left ventricular tissue. Diets were fed for 6 weeks (experiment Table 4.5).
□———□, left ventricular tissue. Diets were fed for 5 weeks (experiment Table 6.5, non-methimazole groups).

4.1.11 Effect on the release of fatty acids from the isolated heart

After feeding diets containing SSO or HCO for 5 days the total fatty acid release from the Langendorff-perfused heart was similar in both groups (about 3 nmol min^{-1}; recalculated from Addendum III, Table V). In another experiment, 8 groups of rats were fed diets containing 35 en% of different types of fat for about 6 weeks. In the working heart, coronary effluent was collected for 1 min periods at $P_{lvf} = 0.80$, 1.06, 1.33 and 1.60 kPa, respectively. The fatty acid releases of the dietary groups were similar, except that of the HSBO group which was somewhat lower (Table 4.6). Table 4.6 shows that the distribution of the various fatty acids released reflects the fatty acid composition of the dietary fats fed.

TABLE 4.6 Release of fatty acids (nmol min^{-1}) from the working heart of rats fed diets containing 35 en% of various fats for 6 weeks.

Fatty acid	Dietary group							
	SSO	SBO	OO	LSO	PO	L	50% SSO + 50% L	86% HSBO + 14% SSO
C 16:0	0.8	1.4	1.0	1.3	1.7	1.9	1.4	0.9
C 18:0	1.1	0.5	0.4	0.5	0.4	0.8	0.7	0.4
C 18:1 (n-9)	1.9	1.4	2.0	1.7	1.7	3.0	1.6	1.4
C 18:2 (n-6)	2.9	1.6	0.9	1.1	0.7	1.1	1.5	0.6
C 18:3 (n-3)	0.8	0.9	0.7	1.3	0.6	0.7	0.7	0.5
C 20:1 (n-9)	0.3	1.1	0.8	0.8	0.6	0.8	0.8	0.5
C 20:4 (n-6)	0.4	0.7	0.4	0.7	0.2	0.1	—	—
Total	8.2	7.6	6.2	7.4	5.9	8.4	6.7	4.3

For abbreviations see Table 2.1; for mean dry heart mass see Table 4.1, experiment e.
Fatty acids were determined in pooled effluents from 7 hearts.

4.1.12 Summary

Feeding rats sunflowerseed oil, in comparison with various other types of dietary fat, for 1 to 6 weeks did not lead to differences in body mass, dry heart mass and spontaneous frequency of the isolated heart; it increased the coronary flow rate in the Langendorff-perfused heart, but, most probably due to the vasodilation caused by the high values of external left ventricular work, not in the working heart. In the working heart, feeding sunflowerseed oil, in comparison with various other fats, increased the maximum external left ventricular work. The effects of sunflowerseed oil on coronary flow rate in the Langendorff-perfused heart and on maximum external left ventricular work in the working heart were found after 1 and 4 weeks of feeding, respectively. Feeding sunflowerseed oil, hydrogenated coconut oil and lard did not

lead to differences in the myocardial oxygen consumption or in the respiratory properties of the isolated heart mitochondria. In the myocardial phospholipids the amount of (n-6) fatty acids increased with the amount of dietary linoleic acid; the amount of arachidonic acid remained almost constant when from 4 to 25 en% linoleic acid was fed.

4.2 TRILINOLEOYLGLYCEROL VERSUS TRIPALMITOYLGLYCEROL

To investigate whether linoleic acid was the cause of the increase in contractility after feeding the SSO-rich diets, two groups of rats (n=11) were fed a diet containing 20 en% trilinoleoylglycerol (TLG) + 5 en% tripalmitoylglycerol (TPG) and 5 en% TLG + 20 en% TPG for 4 weeks, respectively. For convenience the former group will be denoted as the TLG group and the latter one as the TPG group. To the TPG-rich diet a small amount of TLG was added to ensure good digestibility and to prevent essential fatty acid deficiency.

TABLE 4.7 Food uptake and faeces production of rats fed diets containing 20 en% trilinoleoylglycerol (TLG) + 5 en% tripalmitoylglycerol (TPG) or 5 en% TLG + 20 en% TPG.

Dietary group (n = 11)	Food intake /g day^{-1} per rat		Faeces production /g day^{-1} per rat	
	1st week	4th week	1st week	4th week
20 TLG + 5 TPG	15.8 ± 0.3	13.7 ± 0.3	1.95	1.86
5 TLG + 20 TPG	16.8 ± 0.5	16.1 ± 0.6	2.11	2.30
	$P_2 > 0.05$	$P_2 < 0.002$		

Food intake (mean values ± SEM; n = 11) and faeces production (pooled samples) were determined during 2 successive days in the 1st and 4th week of the experimental feeding period.

TABLE 4.8 Body mass (m_b) and dry heart mass (m_h) of rats fed diets containing 20 en% trilinoleoylglycerol (TLG) + 5 en% tripalmitoylglycerol (TPG) or 5 en% TLG + 20 en% TPG for 4 weeks.

Dietary group	m_b/g		m_h/mg
	start	end	
20 TLG + 5 TPG	150 ± 2	299 ± 6	200 ± 4
5 TLG + 20 TPG	149 ± 2	297 ± 6	202 ± 5

Mean values ± SEM; n = 11. Body mass was measured at the start and end (4 weeks) of the experimental feeding period.

To obtain an impression of the digestibility of the diets, food consumption and faeces production were determined at the beginning and at the end of the experiment. The results are listed in Table 4.7 and indicate that the digestibility of the TPG-rich diet was somewhat less than that of the TLG-rich diet. Nevertheless, feeding the two fat mixtures did not result in differences in m_b and m_h of the two groups (Table 4.8).

After the experimental feeding period the heart was isolated and perfused. The values of f_s in the isolated hearts of the two groups did not differ systematically: TLG group 318 ± 8 and TPG group 335 ± 14 min^{-1} ($P_2 > 0.1$).

In the working heart, the upper part of the left ventricular function curve was determined (Fig. 4.4). The results show that feeding the diet rich in linoleic acid increased the contractility of the heart in comparison with the diet low in linoleic acid and rich in palmitic acid. The values of $\dot{Q}_{cor}$, left ventricular output ($\dot{Q}_{lv}$), $\bar{P}_{ao}$ and W_{lv} at four values of P_{lvf} are listed in Table 4.9. This table shows that the higher W_{lv} values of the TLG group were caused by an increase in both $\dot{Q}_{lv}$ and $\bar{P}_{ao}$. As $\bar{P}_{ao}$ is calculated from the peak value of the aortic pressure (P_{aop}) (cf. 2.1.6) and P_{aop} is similar to the maximum left ventricular systolic pressure (P_{lvs}) (cf. Fig. 2.7), this means that P_{lvs} was increased by the linoleic acid-rich diet. The values of $\dot{Q}_{cor}$ of the TLG group are also higher than those of the TPG group. However, for a similar value of W_{lv} the differences are not systematic.

Summarizing: feeding trilinoleoylglycerol, in comparison with tripalmitoylglycerol, to rats for 4 weeks increased the contractility of the working heart; no effects on the other parameters determined were found.

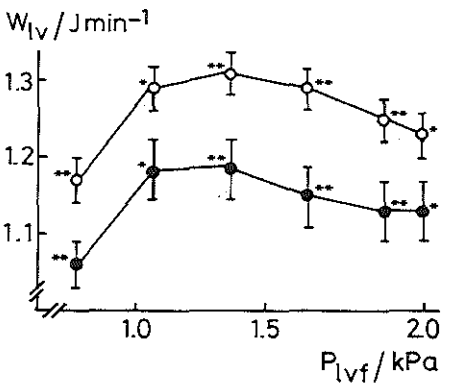

Fig. 4.4 Relationship between left ventricular filling pressure (P_{lvf}) and left ventricular external work (W_{lv}) in the working heart of rats fed 20 en% trilinoleoylglycerol (TLG) + 5 en% tripalmitoylglycerol (TPG) (o———o) or 5 en% TLG + 20 en% TPG (●———●) for 4 weeks. Mean values ± SEM: n = 11; *$P_2 < 0.05$; **$P_2 < 0.02$. For mean dry heart mass see Table 4.8.

TABLE 4.9 Coronary flow rate ($\dot{Q}_{cor}$), left ventricular output ($\dot{Q}_{lv}$), mean aortic pressure ($\bar{P}_{ao}$) and external left ventricular work (W_{lv}) in the working heart of rats fed diets containing 20 en% trilinoleoylglycerol (TLG) + 5 en% tripalmitoylglycerol (TPG) (TLG group) or 5 en% TLG + 20 en% TPG (TPG group) for 4 weeks.

P_{lvf}[1])	$\dot{Q}_{cor}$ / ml min^{-1}		$\dot{Q}_{lv}$ / ml min^{-1}		$\bar{P}_{ao}$ /kPa		W_{lv} / J min^{-1}	
	TLG	TPG	TLG	TPG	TLG	TPG	TLG	TPG
	Group		Group		Group		Group	
0.80	22.0 ± 0.2	20.6 ± 0.6	86.6 ± 1.6	80.4 ± 1.8	14.3 ± 0.1	14.0 ± 0.1	1.17 ± 0.03**	1.06 ± 0.03**
1.07	22.4 ± 0.2	21.4 ± 0.6	98.0 ± 1.8	90.9 ± 2.6	14.3 ± 0.1	14.0 ± 0.1	1.29 ± 0.03*	1.18 ± 0.04*
1.33	22.8 ± 0.2	21.6 ± 0.6	102.0 ± 1.8	94.3 ± 2.8	14.1 ± 0.1	13.9 ± 0.1	1.31 ± 0.03**	1.18 ± 0.04**
1.60	22.6 ± 0.2	21.6 ± 0.6	102.6 ± 1.8	94.5 ± 3.0	14.1 ± 0.1	13.7 ± 0.1	1.29 ± 0.03**	1.15 ± 0.04**

Mean values ± SEM; n = 11. TLG vs. TPG: *$P_2 < 0.05$, **$P_2 < 0.02$.
[1]) left ventricular filling pressure.
For mean dry heart mass see Table 4.8.

4.3 RELATIONSHIP BETWEEN THE AMOUNT OF DIETARY LINOLEIC ACID AND MAXIMAL EXTERNAL LEFT VENTRICULAR WORK

4.3.1 Introduction

In section 4.1 it has been described that after feeding rats diets containing SSO, $W_{lv}(max)$ in their isolated hearts is higher than after feeding diets containing saturated fats like HCO or L. In section 4.2 it has been made plausible that the increase in $W_{lv}(max)$ by the SSO-rich diets is caused by the linoleic acid present in SSO. Therefore, the relationship between the amount of dietary linoleic acid and $W_{lv}(max)$ was studied. As index of the amount of dietary linoleic acid the dietary ratio of linoleic acid and saturated fatty acids (LA/SFA) is used to correct for differences in the total amount of dietary fat between the various experiments. Moreover, it may be possible that the effect of the amount of linoleic acid is influenced by the amount of saturated fatty acids as in the case of the effect of the amount of linoleic acid on serum cholesterol [1,4]. In the evaluation of the relationship between LA / SFA and $W_{lv}(max)$ only experiments in which SSO, L or mixtures of SSO and L were fed have been considered, because too few data on other dietary fats were available. Data from experiments already described in section 4.1 and from two additional experiments will be used. In total six experiments are taken into account which have been listed in Table 3.1. First, details of the two experiments not yet described will be presented.

4.3.2 Additional experiments

In two experiments, numbered 5 and 6, groups of rats were fed mixtures of SSO and L for 5-6 weeks. The mixtures fed are listed in Table 4.10. This table shows that feeding the mixtures did not result in systematic differences in m_b and m_h. For the fatty acid composition of the myocardial phospholipids (experiment 5) see Table 4.5. In the working heart, the relationship between P_{lvf} and W_{lv} was determined. P_{aoh} in the experiments 5 and 6 was 9.3 and 13.6 kPa, respectively. A higher value of P_{aoh} was used in experiment 6 to investigate whether P_{aoh} affects the relationship between LA / SFA and $W_{lv}(max)$. At $P_{aoh} = 9.3$ kPa, $W_{lv}(max)$ of all the groups was obtained at $P_{lvf} = 1.33$ kPa, but at $P_{aoh} = 13.6$ kPa, $W_{lv}(max)$ was obtained at $P_{lvf} = 1.46$ kPa. The mean values of $W_{lv}(max)$ and the corresponding values of $\dot{Q}_{cor}$, $\dot{Q}_{lv}$ and $\bar{P}_{ao}$ are listed in Table 4.11. This table shows that as a result of the higher value of $\bar{P}_{aoh}$, higher values of $W_{lv}(max)$ were obtained which were caused by an increase in $\bar{P}_{ao}$ and not in $\dot{Q}_{lv}$, which is the usual effect of an increase in after-load.

TABLE 4.10 Body mass (m_b) at the start and the end of the experimental feeding period, and dry heart mass (m_h) of rats fed mixtures (total 35 en%) of sunflowerseed oil (SSO) and lard (L) for 5-6 weeks.

Experiment[1])	Dietary group	m_b/g		m_h/mg
		start	end	
5	10 SSO + 25 L	215 ± 5	327 ± 8	213 ± 4
	15 SSO + 20 L	221 ± 5	333 ± 10	214 ± 8
	20 SSO + 15 L	219 ± 4	325 ± 7	213 ± 5
	25 SSO + 10 L	218 ± 6	336 ± 8	226 ± 5
6	6 SSO + 29 L	228 ± 3	339 ± 8	214 ± 6
	21 SSO + 14 L	226 ± 2	329 ± 6	217 ± 4
	28 SSO + 7 L	227 ± 3	342 ± 6	218 ± 3
	32 SSO + 3 L	226 ± 3	330 ± 6	221 ± 4
	35 SSO	226 ± 3	330 ± 6	207 ± 4

Mean values ± SEM; n = 11.
[1]) Experiments 5 and 6 are the same as in Table 3.1.

4.3.3 Relationship between the dietary ratio linoleic acid / saturated fatty acids and maximal external left ventricular work

In chapter 3 it has been shown that $W_{lv}(max)$ depends on m_h. As m_h in the six experiments varies to some extent (Table 3.1) the mean values of $W_{lv}(max)$ of the groups have been adjusted to $m_h = 220$ mg (the mean value of m_h in the six experiments) by means of the analysis of covariance. For the six experiments, the relationships between LA/SFA and the adjusted values of $W_{lv}(max)$ are shown in Fig. 4.5. This figure shows clearly that $W_{lv}(max)$ is systematically related to LA/SFA.

W_{lv} is calculated from $\dot{Q}_{lv}$ and P_{aop}. Therefore, LA/SFA will also be related to these parameters, at least to one of them. Just for the sake of illustration (statistical evaluation has not been performed), the relationships between LA/SFA and $\dot{Q}_{lv}$ as well as P_{aop} are also shown in Fig. 4.5. The figure indicates that both $\dot{Q}_{lv}$ and P_{aop} are related to LA/SFA.

4.3.4 Summary

In the isolated, working rat heart the maximal value of the external left ventricular work was positively, linearly related to the ratio of dietary linoleic acid and saturated fatty acids.

TABLE 4.11 Coronary flow rate ($\dot{Q}_{cor}$), cardiac output ($\dot{Q}_{lv}$), mean aortic pressure ($\bar{P}_{ao}$) and maximal external left ventricular work ($W_{lv}(max)$) in the working heart of rats fed mixtures (total 35 en%) of sunflowerseed oil (SSO) and lard (L) for 5-6 weeks.

Experiment	Dietary group	P_{aoh} [1] /kPa	P_{lvf} [2] /kPa	$\dot{Q}_{cor}$ /ml min^{-1}	$\dot{Q}_{lv}$ /ml min^{-1}	$\bar{P}_{ao}$ /kPa	$W_{lv}(max)$ /J min^{-1}
5	10 SSO + 25 L	9.3	1.33	25.1 ± 0.9	96.9 ± 1.9	13.9 ± 0.1	1.21 ± 0.03
	15 SSO + 20 L			25.3 ± 0.6	96.5 ± 2.4	14.0 ± 0.1	1.22 ± 0.03
	20 SSO + 15 L			24.9 ± 0.9	97.9 ± 2.3	14.1 ± 0.1	1.25 ± 0.03
	25 SSO + 10 L			27.1 ± 0.7	101.5 ± 1.6	14.3 ± 0.1	1.31 ± 0.03
6	6 SSO + 29 L	13.6	1.46	33.6 ± 1.3	96.9 ± 1.7	18.0 ± 0.1	1.61 ± 0.05
	21 SSO + 14 L			33.2 ± 0.7	95.5 ± 2.2	18.5 ± 0.2	1.62 ± 0.04
	28 SSO + 7 L			34.0 ± 0.9	100.5 ± 2.8	18.3 ± 0.1	1.69 ± 0.04
	32 SSO + 3 L			36.5 ± 1.5	101.0 ± 2.0	18.4 ± 0.2	1.71 ± 0.04
	35 SSO			34.4 ± 0.4	97.9 ± 1.6	18.3 ± 0.1	1.64 ± 0.04

Mean values ± SEM; n = 9.

[1]) Hydrostatic aortic pressure.

[2]) Left ventricular filling pressure.

The adjusted values of $W_{lv}(max)$ calculated for dry heart mass = 220 mg are listed in Table 3.1. For additional data see Table 4.10.

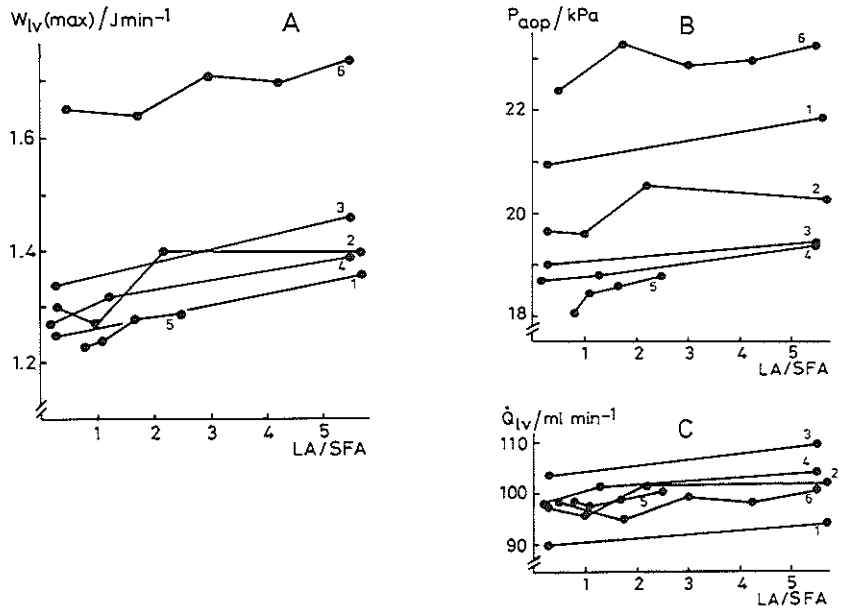

Fig. 4.5 Relationship between the dietary ratio of linoleic acid and saturated fatty acids (LA / SFA) and maximal external left ventricular work (W_{lv}(max)), (A), peak aortic pressure (P_{aop}) (B) and cardiac output ($\dot{Q}_{lv}$) (C), respectively.

The numbers 1-6 refer to the experiments listed in Table 3.1. Since a relationship exists between dry heart mass (m_h) and W_{lv}(max), and m_h between the various experiments differed to some extent, W_{lv} (max) was adjusted to $m_h = 220$ mg by analysis of covariance (cf. chapter 3). For the experiments 1-4 and experiment 6 analysis of variance showed that W_{lv}(max) is related to LA / SFA ($P < 0.001$). For the 5 experiments the regression coefficients of the relationship between LA / SFA and W_{lv}(max) are similar (range from 0.019 ± 0.008 to 0.023 ± 0.011; mean values ± SD). In the analysis of variance experiment 5 was omitted because of the narrow LA / SFA range (regression coefficient 0.037 ± 0.022). The relationships between LA / SFA and P_{aop} as well as $\dot{Q}_{lv}$ are also shown but no statistical evaluation of these relationships was performed. The mean values of P_{aop} and $\dot{Q}_{lv}$ were also adjusted to $m_h = 220$ mg. Mean values of W_{lv}(max), P_{aop} and $\dot{Q}_{lv}$; for the SEM of W_{lv}(max) see Table 3.1.

4.4 EFFECT OF DIETARY FAT ON THE RELATIONSHIP BETWEEN HEART FREQUENCY AND EXTERNAL LEFT VENTRICULAR WORK

In all experiments with the working heart, the heart was electrically paced at $f_p = 360\ min^{-1}$. This frequency was chosen since in preliminary experiments it had been found that the highest value of $W_{lv}(max)$ was obtained at this frequency. The effect of the heart frequency on $W_{lv}(max)$ is related to the relationship between heart frequency and contractile force (staircase effect). In the Langendorff-perfused rat heart, by other authors, a maximum in the relation between f_p and contractile force was found at $f_p = 480\ min^{-1}$, but in other heart preparations other relations were observed [2]. For the working rat heart no data were available.

TABLE 4.12 External left ventricular work (W_{lv}) and coronary flow rate ($\dot{Q}_{cor}$) at 4 values of left ventricular filling pressure (P_{lvf}) in the working heart of rats fed 35 en% sunflowerseed oil (SSO) or 33 en% lard + 2 en% SSO for 5 weeks.

P_{lvf} /kPa	W_{lv} / J min^{-1}		$\dot{Q}_{cor}$ / ml min^{-1}	
	Group		Group	
	35 SSO	33 L + 2 SSO	35 SSO	33 L + 2 SSO
0.80	1.26 ± 0.05*	1.10 ± 0.04*	24.5 ± 0.6	23.6 ± 0.8
1.07	1.42 ± 0.04*	1.28 ± 0.04*	26.5 ± 0.6	25.1 ± 0.6
1.33	1.43 ± 0.03*	1.33 ± 0.03*	26.7 ± 0.6	25.7 ± 0.6
1.60	1.38 ± 0.03*	1.29 ± 0.03*	26.5 ± 0.6	25.5 ± 0.8

Mean values ± SEM; n = 9.
35 SSO vs 33 L + 2 SSO: * $P_2 < 0.05$.

TABLE 4.13 Effect of pacing frequency (f_p) on external left ventricular work (J min^{-1}; mean values ± SEM, n = 3) in the working heart of rats fed 35 en% sunflowerseed oil (SSO) or 33 en% lard + 2 en% SSO for 5 weeks.

Dietary group	f_p/min^{-1}		
	360	400	360
35 SSO	1.41 ± 0.08	1.30 ± 0.09	1.37 ± 0.07
33 L + 2 SSO	1.25 ± 0.04	1.13 ± 0.03	1.22 ± 0.07

Pacing frequency was increased from 360 to 400 min^{-1} after which it was decreased to 360 min^{-1}.
For the two groups: 360 min^{-1} vs 400 min^{-1}, $P_2 < 0.001$; 400 min^{-1} vs 360 min^{-1}, $P_2 < 0.002$.

It cannot be excluded that the type of dietary fat affects the relationship between f_p and W_{lv}, and as the function curves in the working rat heart were determined at constant frequency (f_p = 360 min^{-1}), this might have affected the function curves and thus W_{lv}(max) of the dietary groups. To test this, 2 groups of rats were fed diets containing 35 en% SSO or 33 en% L + 2 en% SSO for 5 weeks, and in the working heart the relationship between f_p and W_{lv} was determined (cf. 2.1.8) at a constant value of P_{lvf}. For this, the value of P_{lvf} was chosen at which W_{lv}(max) was obtained when the hearts were stimulated at f_p = 360 min^{-1}. To determine this value of P_{lvf}, W_{lv} was first determined at 4 values of P_{lvf} (Table 4.12). As it was found that W_{lv}(max) was attained at P_{lvf} = 1.33 kPa, P_{lvf} was kept at this value, after which the relationship between f_p and W_{lv} was determined. Fig. 4.6 shows that the relationships for the two groups are similar. Moreover, the highest value of W_{lv} was obtained at f_p = 360 min^{-1}. The relationship between f_p and stroke work is shown in Fig. 4.7. To test whether the decrease in W_{lv} at f_p >360 min^{-1} was due to deterioration of the heart, in 3 hearts of each group f_p was decreased from 400 to 360 min^{-1}. The results (Table 4.13) show that W_{lv} increased again but remained somewhat below the first determination at f_p = 360 min^{-1}.

The mean values of m_b and m_h of the two groups are listed in Table 4.14 and show that also in this experiment the dietary fats did not affect these parameters.

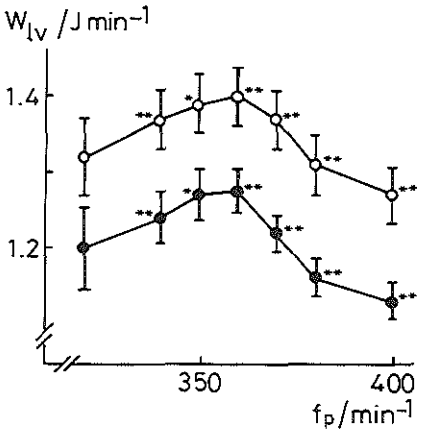

Fig. 4.6 Relationship between pacing frequency of the heart (f_p) and external left ventricular work (W_{lv}) in the working heart of rats fed 35 en% sunflowerseed oil (SSO) (o——o) or 33 en% lard + 2 en% SSO (●——●) for 5 weeks.

Left ventricular filling pressure was 1.33 kPa. Mean values ± SEM; n = 9, except for f_p = 320 min^{-1} where n = 3. Between the groups: * P_2 < 0.05; ** P_2 < 0.02. Within the groups: 340 min^{-1} vs 360 min^{-1}, P_2 < 0.01. For mean dry heart mass see Table 4.14.

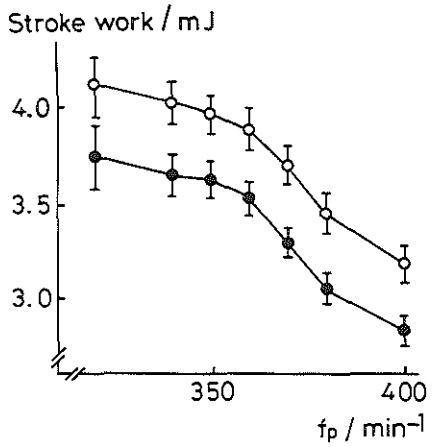

Fig. 4.7 Relationship between pacing frequency (f_p) and stroke work in the working heart of rats fed 35 en% sunflowerseed oil (SSO) (o———o) or 33 en% lard + 2 en% SSO (●———●) for 5 weeks.
Mean values ± SEM; n = 9, except for $f_p = 320$ min^{-1} where n = 3.

TABLE 4.14 Body mass (m_b) at the start and end of the experimental feeding period, and dry heart mass (m_h) of rats fed 35 en% sunflowerseed oil (SSO) or 33 en% L + 2 en% SSO for 5 weeks.

Dietary group	m_b/g		m_h/mg
	start	end	
35 SSO	232 ± 3	335 ± 3	210 ± 4
33 L + 2 SSO	231 ± 3	330 ± 5	209 ± 4

Mean values ± SEM; n = 9.

Summarizing: feeding rats diets containing sunflowerseed oil or lard did not result in a difference in the relationship between heart frequency and external left ventricular work in their isolated, working hearts.

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5 ROLE OF PROSTAGLANDINS IN THE DIETARY FAT-INDUCED CHANGES IN THE ISOLATED RAT HEART

5.1 INTRODUCTION

The preceding chapter has shown that the increases in coronary flow rate ($\dot{Q}_{cor}$) and external left ventricular work (W_{lv}) or maximal external left ventricular work ($W_{lv}(max)$) found after feeding linoleic acid-rich diets in comparison with linoleic acid-low diets, were caused most probably by the linoleic acid. In the body, linoleic acid is used for energy production, it is incorporated into phospholipids (PL) and cholesteryl esters and it is elongated and desaturated to a number of (n-6) polyunsaturated fatty acids such as C20:3(n-6), dihomo- γ -linolenic acid, and C20:4(n-6), arachidonic acid, of which arachidonic acid is by far the most important one. These polyunsaturated fatty acids are incorporated into the PLs of the membranes. For prostaglandin (PG) synthesis dihomo-γ-linolenic and arachidonic acid are set free by phospholipase A_2 [14], and, as recently found, by phospholipase C and a diacylglycerol lipase from phosphatidylinositol [4]. PGs of the 1-series are synthesized from dihomo- γ -linolenic acid and PGs of the 2-series from arachidonic acid.

In most tissues, the amount of dihomo- γ -linolenic acid in total PL or other lipid fractions is very low (<1%), whereas arachidonic acid is abundantly present (up to 45%) [8, 10, 32]. In the myocardial PLs, we found that by feeding more than 3 en% linoleic acid the amount of arachidonic acid was about 24%, whereas dihomo- γ -linolenic acid could not or hardly be determined (cf. Addenda III (Table VI) and VI (Table VII); Tables 4.5 and 6.4). In essential fatty acid (EFA) deficiency, dihomo- γ -linolenic acid is not present in detectable amounts in tissue lipids, whereas arachidonic acid remains present [8, 40]. The amounts of dihomo- γ -linolenic and arachidonic acid occurring in the PLs indicate that PGs of the 2-series will be more important than PGs of the 1-series, and indeed in almost every tissue or cell type, synthesis of PGs of the 2-series has been found, whereas synthesis of PGs of the 1-series has been found only in a few cases [9]. PGs of the 1-series may have been "neglected" by this dominant role of the PGs of the 2-series: certain effects of dihomo-γ -linolenic acid have been found which differ from those of arachidonic acid

[32, 34, 37]. Nevertheless, the present investigation was only directed to PGs of the 2-series, because in the gas chromatographic determinations no indications were found of the occurrence of PGs of the 1-series in the heart perfusates (D.H. Nugteren, personal communication).
As dietary linoleic acid is the ultimate precursor of the PGs of the 2-series and as PGs are very potent vasodilators and some PGs increase myocardial contractile force under certain conditions (cf. Addendum VI), the increase in $\dot{Q}_{cor}$ and $W_{lv}(max)$ as found in the isolated rat heart after feeding linoleic acid-rich diets in comparison with linoleic acid-low diets might have been caused by an increase in the PG synthesis in the isolated heart. Therefore, the effect of feeding various dietary fats on the PG release from the isolated rat heart, and also on that from the isolated rabbit heart, was investigated. Moreover, the role of PGs in the regulation of $\dot{Q}_{cor}$ and W_{lv} in the isolated rat heart was studied. Most results have already been published in the Addenda V-VII. In this chapter the main results are presented together with some additional data. Since no relationship was found between dry heart mass (m_h) and PG release (cf. 5.3), and feeding the dietary fats did not affect m_h (cf. chapter 3), in this chapter the PG release is not expressed per unit of m_h as done in the Addenda.

5.2 INFLUENCE OF TYPE OF DIETARY FAT ON PG SYNTHESIS IN THE ISOLATED RAT AND RABBIT HEART AND ISOLATED RAT AORTA

5.2.1 Introduction

At the time when the study on the influence of dietary fat on PG production in the isolated heart was started, only a few data about the effect of dietary fat on PG production were available. It had been established that in EFA deficiency, PG synthesis decreased in the rabbit kidney [12] and in adipose tissue [8]. That there is still some PG production is caused by the fact that arachidonic acid is always present in the PLs of the membranes of EFA deficient animals [17]. Moreover, it has been found that in EFA deficiency the cyclooxygenase activity in homogenates of rat kidney medulla is increased when incubated with exogenous arachidonic acid [24]. However, Ziboh et al. [41] have found that eicosatrienoic acid (20:3(n-9)), which is present in the PLs of EFA deficient animals, is an inhibitor of cyclooxygenase as a result of which the cyclooxygenase activity might be inhibited when this fatty acid is set free from the PLs. In the rat, feeding linoleic acid-rich diets increased the plasma level of PGs [20] and feeding the PG precursor dihomo-γ-linolenate increased the urinary excretion of PG metabolites [10, 32]. Also, by feeding a linoleic acid-rich diet in comparison with a diet containing rapeseed oil, the ad-

renocorticotrophin-stimulated PG synthesis in the rat adrenal was increased [7].

These data show that the amount of dietary linoleic acid can increase PG synthesis. Therefore, it was investigated whether feeding diets containing sunflowerseed oil (SSO), lard (L) or hydrogenated coconut oil (HCO) affects the PG synthesis in the isolated heart under the conditions as used for the determination of $\dot{Q}_{cor}$ and W_{lv}.

5.2.2 Prostaglandin and thromboxane release from the Langendorff-perfused heart and isolated rat aorta

Groups of rats and rabbits were fed for 4 weeks experimental diets (cf. 2.3.2) containing 35 and 40%, respectively, of the total digestible energy (en%) as fat. The fats were SSO, L and HCO. The groups are denoted by the amount of fat fed and the abbreviation of the fat. After the feeding period the hearts were isolated and perfused according to the modified Langendorff technique (cf. 2.1.3); perfusion pressure (P_p) 8.0 kPa (rabbit heart) or 9.3 kPa (rat heart), stimulation frequency (f_p) 250 min^{-1} (rabbit heart) or 360 min^{-1} (rat heart). Twenty minutes after the start of perfusion (t = 20 min), perfusates were collected for 10 or 15 min. Determination from t = 20 min was chosen because the preparation of the heart for the perfusion according to the modified Langendorff technique takes about 10 min and because PG synthesis might have been stimulated as a result of the preparation. At t = 20 min PG release is about constant and remains at the same level or declines somewhat during the perfusion (cf. Addendum II). This PG release is called the basal PG release. PGs were determined in pooled perfusates by gas chromatography (cf. 2.2.1.1). For the determination of the PGI_2 release from the isolated aorta, a piece of the thoracic aorta was isolated (together with the heart) and perfused (cf. 2.2.6). In the perfusates, PGI_2 was determined using the rat platelet bioassay (cf. 2.2.1.2).

Table 5.1 shows that after feeding rabbits and rats diets containing SSO, in comparison with diets containing L or HCO, the basal PG release from their isolated hearts and also from the isolated rat aorta tended to be lower. The table also shows that thromboxane (TX) B_2 which is the decomposition product of TXA_2, was released from the isolated heart; this release tended also to be somewhat lower in the SSO groups. The TX release is further discussed in section 5.4. From Table 5.1, the releases of the various PGs and TXB_2, in percents of the sum of the PGs and TXB_2 releases, were calculated (Table 5.2). These data demonstrate that PGI_2, of which 6-oxo-$PGF_{1\alpha}$ is the decomposition product, is the main PG released from the heart.

The modified Langendorff technique, in which the interstitial effluent is collected separately from the venous effluent, was used to get an impression

TABLE 5.1 Influences of feeding various dietary fats to rats and rabbits for 4 weeks on coronary flow rate ($\dot{Q}_{cor}$) and prostaglandin (PG) and thromboxane (TX) release in their isolated hearts, and on the release of PGI_2 from isolated rat aorta.

Preparation	Dietary group	m_h[1])/mg	$\dot{Q}_{cor}$[2])/ ml min^{-1}	Release of TX and PGs/pmol min^{-1}						PGI_2
				TXB_2	PGD_2	PGE_2	$PGF_{1\alpha}$	6-oxo-$PGF_{1\alpha}$	Total PGs	/pmol $min^{-1}cm^{-2}$
Rabbit heart	40 SSO	687 ± 41	31.1 ± 2.1	n.d.	n.d.	3.3	1.7	14.7	19.7	—
	37 L + 3 SSO	616 ± 56	32.9 ± 1.9	n.d.	n.d.	5.1	1.9	24.7	31.7	—
Rat heart	35 SSO	210 ± 4	18.7 ± 0.5	0.4	1.4	1.6	0.5	5.6	9.1	—
	33 L + 2 SSO	217 ± 6	17.6 ± 0.6	0.7	0.9	1.4	0.5	9.9	12.7	—
	30 HCO + 5 SSO	208 ± 3	16.4 ± 0.9	0.8	0.7	1.4	0.4	11.0	13.5	—
	35 SSO	212 ± 5	19.1 ± 0.9[3])	0.3	0.5	1.9	0.5	4.5	7.4	—
	30 HCO + 5 SSO	211 ± 4	15.2 ± 1.0[3])	0.4	0.7	2.0	0.6	6.0	9.2	—
Rat aorta	35 SSO	—	—	—	—	—	—	—	—	1.1 ± 0.14
	30 HCO + 5 SSO	—	—	—	—	—	—	—	—	1.4 ± 0.14

PGs, except PGI_2 and TXB_2 were determined in pooled effluents from 7 hearts by gas chromatography; n.d., not determined. PGI_2 (mean values ± SEM; n = 7) was determined using the rat platelet bio-assay. 40 SSO: 40 en% sunflowerseed oil, etc. (cf. Table 2.1).
Summary of the results shown in Addendum V, Tables 1, 2 and 5. TXB_2 release and the releases of PGs are the sum of the amounts released into the interstitial and venous effluents from the modified Langendorff-perfused heart. The amount of 6-oxo-$PGF_{1\alpha}$ was multiplied by a factor of 2.5 (cf. 2.2.1.1).

[1]) Dry heart mass; mean values ± SEM, n = 7.

[2]) Average value during the collection of the effluents; mean values ± SEM, n = 7.

[3]) $P_2 < 0.02$.

TABLE 5.2 Amounts (in % of the total amount) of prostaglandins (PG) and thromboxane (TX) released from the isolated rabbit and rat heart.

PG, TX	Rabbit heart		Rat heart	
	Control [1]	Anoxia[2]	Langendorff[1]	Working[3]
6-oxo-$PGF_{1\alpha}$	77	73	68	74
PGE_2	16	11	15	20
PGD_2	n.d.	3	8	n.d.
$PGF_{2\alpha}$	7	6	4	6
TXB_2	n.d.	7	5	n.d.

[1], [2] and [3] Calculated from data in the Tables 5.1, 5.4 and 5.3, respectively. n.d., not determined.

about the location of the PG (or TX) release. Assuming that PGs are released from various myocardial tissues, the interstitial effluent will contain PGs from the myocardial wall (coronary vessels, muscle cells, etc.) and the heart surface, whereas the venous effluent will contain PGs from the coronary vessels, to some extent from other parts in the myocardial wall, and from the linings of the right atrium and ventricle and pulmonary artery. In addition, both effluents will contain PGs from the piece of aorta between the aortic cannula and the aortic valves and from the lining of the left ventricle (in the Langendorff-perfused heart a small amount of fluid may be ejected by the left ventricle). Because of all these possible places of PG release it is very difficult to locate PG release. Only when PGs are present in the venous and interstitial effluents, which always was the case (cf. Addendum V), it is likely that the coronary vessels are a site of PG synthesis. Moreover, cultured endothelial cells synthesize PGI_2 [26], whereas it has been found that cultured rat heart myocytes do not [1]. On the other hand, human lymphatics [27] and the surface of the rat heart (De Deckere, unpublished results) also synthesize PGI_2.

5.2.3 Prostaglandin release from the working rat heart

Eight groups of rats (cf. 4.1.5) were fed experimental diets (cf. 2.3.2) containing 35 en% of 8 different types of fat for about 6 weeks, after which the hearts were isolated and perfused as working hearts (cf. 2.1.4). At left ventricular filling pressures of 0.80, 1.06, 1.33 and 1.60 kPa, coronary effluent was collected for 1 min periods. In the pooled effluents from the hearts of each dietary group PGs were determined by gas chromatography (cf. 2.2.1.1).
Feeding linseed oil (LSO) and hydrogenated soyabean oil (HSBO) resulted in a low PG release, whereas after feeding sunflowerseed oil, soyabean oil, olive oil, palm oil and lard the PG releases were higher and quite similar (Table 5.3). The inhibitory effect of feeding LSO on the PG release, which has al-

so been found in other rat tissues [18,21], is caused by the fact that linolenic acid is desaturated and elongated in preference to linoleic acid. Consequently, eicosapentaenoic acid (20:5(n-3)) is formed [19, 21] which is incorporated into the PLs on the place of arachidonic acid. As eicosapentaenoic acid is a poor substrate for cyclooxygenase, as compared to arachidonic acid [31], the result will be a decrease in prostaglandin synthesis.

TABLE 5.3 Release of prostaglandins (PG; pmol min^{-1}) from the working heart of rats fed diets containing 35 en% of various fats for 6 weeks.

PG	Dietary group							
	SSO	SBO	OO	LSO	PO	L	50% SSO + 50% L	86% HSBO + 14% SSO
PGE_2	3.1	2.0	2.8	1.1	2.5	2.6	1.7	0.9
$PGF_{2\alpha}$	0.6	0.9	0.7	0.5	0.7	0.5	0.6	0.6
6-oxo-$PGF_{1\alpha}$	7.5	11.8	8.9	3.9	7.3	8.2	8.0	5.0
Total	11.2	14.7	12.4	5.5	10.5	11.3	10.3	6.5

Coronary effluents were collected at various values of left ventricular filling pressure for 1 min periods. PGs were determined in pooled effluents from 7 hearts by gas chromatography. For abbreviations see Table 2.1. For mean dry heart mass see Table 4.1.

The lower PG release after feeding HSBO has not been investigated further, but the total amount of fatty acids released from the hearts of the HSBO group was also rather low in comparison with the amount found for the other 7 groups (cf. Table 4.6).

The similarity between the values of the PG release from the Langendorff-perfused (Table 5.1) and working heart (Table 5.3) shows that the stronger movement and the stronger pulsations in the coronary vessels in the working heart, in comparison with the Langendorff-perfused heart, did not stimulate PG synthesis.

5.2.4 Prostaglandin release from the Langendorff-perfused rabbit heart after anoxia

The previous sections have shown that feeding the various fats used affected the basal PG released only in slight measure (except LSO). It is possible that when PG synthesis is stimulated, distinct differences in the PG release between the dietary groups appear. Therefore, PG release was determined after a short period of anoxia, which is a well known stimulus of PG synthesis [5, 6, 29]. Two groups of rabbits were fed experimental diets (cf. 2.3.2) containing 40 en% SSO or 37 en% L + 3 en% SSO for 4 weeks after which the hearts were

isolated and perfused according to Langendorff (cf. 2.1.2). After an anoxic period of 3 min, perfusates were collected for 10 min. In the pooled perfusates of each group, PGs and TXB_2 were determined by gas chromatography (cf. 2.2.1.1). The values of the PGs and TXB_2 releases of the two groups (Table 5.4) show that feeding the 2 dietary fats did not lead to systematic differences in the PG and TX synthesis in the heart after anoxia. Comparison of the total PG release after anoxia with the total PG release under control conditions (Table 5.1) shows that the PG release immediately after anoxia was about twice that under control conditions.

TABLE 5.4 Release (pmol min^{-1}) of thromboxane (TX) and prostaglandins (PG), following anoxia, from the Langendorff-perfused heart of rabbits fed diets containing 40 en% sunflowerseed oil (SSO) or 37 en% lard (L) + 3 en% SSO for 4 weeks.

Group (n=8)	m_h[1])/mg	TXB_2	PGD_2	PGE_2	$PGF_{2\alpha}$	6-oxo-$PGF_{1\alpha}$	Total PGs
40 SSO	677 ± 28	4.3	1.7	6.8	3.5	45.0	57.0
37 L + 3 SSO	718 ± 32	4.3	2.5	8.0	3.7	50.1	64.3

From t = 15-18 min the perfusion fluid was gassed with 95% N_2 + 5% CO_2 to induce anoxia after which the perfusion fluid was reoxygenated (95% O_2 + 5% CO_2). Perfusates for the determination of TX and PGs were collected from t = 18-28 min. In pooled samples, TX and PGs were determined by gas chromatography. Perfusion pressure 8.0 kPa; hearts were not stimulated. Before anoxia coronary flow rate was about 38 ml min^{-1} and spontaneous heart frequency about 219 min^{-1} (both groups). These values were also obtained 10 min after the anoxic period.
[1]) Dry heart mass; mean values ± SEM.

Isolation of the heart is also a stimulus of PG synthesis, most probably, because during isolation an ischemic period of about 1 min occurs. In the next section, the effect of feeding various dietary fats on PG synthesis immediately after the isolation of the heart is described.

5.3 TIME COURSE OF PGI_2 RELEASE, AND EFFECT OF INHIBITION OF PG SYNTHESIS ON CORONARY FLOW RATE IN THE LANGENDORFF-PERFUSED HEART OF RATS FED VARIOUS DIETARY FATS

In section 5.2.2 it has been shown that there were no differences in the basal PG releases by which the increase in $\dot{Q}_{cor}$ found after feeding linoleic acid-rich diets in comparison with linoleic acid-low diets (cf. chapter 4) could be explained. This does not mean that PGs could not be involved. For instance, it might be that as a result of the isolation of the heart (stimulation of PG synthesis) the PG synthesis at the beginning of the perfusion is higher in the hearts of the SSO-fed rats than in the hearts of the saturated fat-fed rats,

as a result of which $\dot{Q}_{cor}$ during the control perfusion circumstances is increased. When this is the case then complete inhibition of myocardial PG synthesis during isolation and perfusion will result in similar values of $\dot{Q}_{cor}$ for the various dietary fat groups.

Three groups (n=20) of rats were fed diets containing 35 en% SSO, 35 en% L or 30 en% HCO + 5 en% SSO for 4 weeks. At the end of the experimental feeding period body mass in each of the 3 groups was about 320 g. One and a half hours before isolation of the heart 10 rats of each group were injected with 1.5 ml saline (placebo) and the other 10 rats with a solution of acetylsalicylic acid (ASA). To this end, about 27 μmol ASA was suspended and partly dissolved in 1.5 ml saline after which about 27 μmol NaOH was added as a result of which ASA completely dissolved. Within a few min after the adition of NaOH, the solution was injected intraperitoneally. As a result of this treatment with ASA, PG synthesis in the isolated heart is completely inhibited (cf. Addendum VII). In the Langendorff-perfused heart (P_p = 9.3 kPa) $\dot{Q}_{cor}$ was determined every min, and at t = 2, 5, 10, 15, 20 and 30 min coronary effluent was collected during 0.5 min to determine the PGI_2 release. The hearts were stimulated (f_p = 360 min^{-1}) from t = 3 min on.

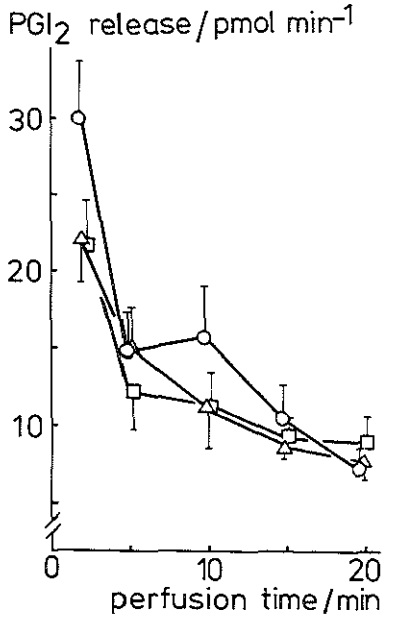

Fig. 5.1 Time courses of PGI_2 release in the Langendorff-perfused heart (placebo) of rats fed various fats for 4 weeks.
(o———o) 35 en% sunflowerseed oil (SSO); (□———□) 35 en% lard; (△———△) 30 en% hydrogenated coconut oil + 5 en% SSO. For dry heart mass see Table 5.6. Mean values ± SEM; n = 9-10.

In the first 10 min of perfusion the PGI_2 release from the control hearts (placebo) of the SSO group was higher than from the hearts of the other 2 fat groups, but after 15 min of perfusion the PGI_2 releases in the 3 groups were similar (Fig. 5.1). The average PGI_2 releases during t = 2-10 min in the SSO, L and (30 HCO + 5 SSO) groups were 20.3 ± 2.4, 16.0 ± 1.4 and 15.0 ± 1.3 pmol min^{-1}, respectively. Thus, a tendency for a somewhat higher PGI_2 release in the SSO group was found at the beginning of perfusion, but the results do not differ systematically. In the hearts of the ASA-treated rats, PGI_2 release at t = 2 min was not detectable (<3 pmol min^{-1}). The time courses of $\dot{Q}_{cor}$ are shown in Fig. 5.2. Those of the 3 placebo groups differ, but the values of $\dot{Q}_{cor}$ did not differ systematically at any moment; the time courses of $\dot{Q}_{cor}$ of the 3 ASA groups are similar.

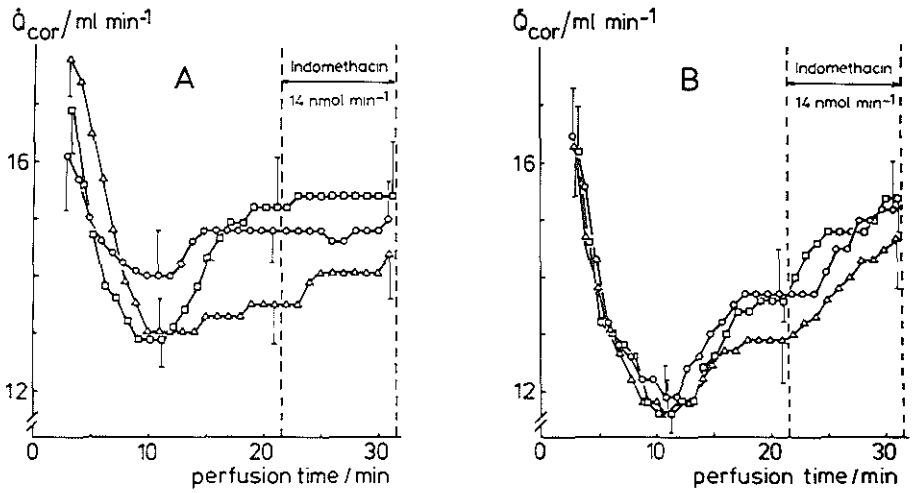

Fig. 5.2 Time courses of coronary flow rate ($\dot{Q}_{cor}$) in the Langendorff-perfused heart of rats fed various fats for 4 weeks.
Ninety min before isolation of the heart, rats were injected intraperitoneally with 1.5 ml saline (A) or with a solution of acetylsalicylic acid (B) (cf. 5.3). At t = 21 min a solution of indomethacin (cf. Addendum VII) was added to the perfusion fluid above the heart resulting in an indomethacin concentration (at t = 21) of about 1.0 (A) and 1.1 nmol ml^{-1} (B). Mean values (n = 9-10); SEM values ranged from 4 to 6%. The differences in $\dot{Q}_{cor}$ between the 3 groups (both in A and B) are not systematic. Perfusion pressure 9.3 kPa. (o———o) 35 en% sunflowerseed oil (SSO); (□———□) 35 en% lard; (△———△) 30 en% hydrogenated coconut oil + 5 en% SSO. For dry heart mass see Table 5.6.

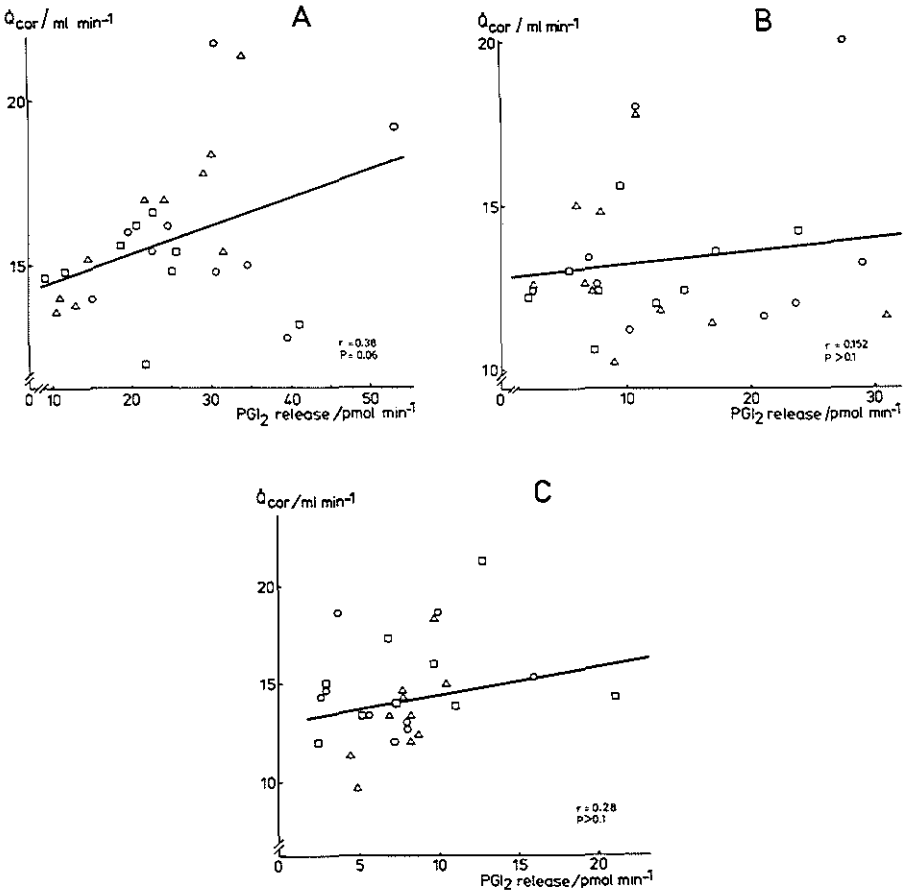

Fig. 5.3 Relationship between PGI_2 release and coronary flow rate ($\dot{Q}_{cor}$) in the Langendorff-perfused rat heart.
Three groups of rats were fed various dietary fats (see legend to Fig. 5.1) for 4 weeks. In the isolated heart, PGI_2 release and $\dot{Q}_{cor}$ were determined at t = 2-3 min (A), t = 10-11 min (B) and t = 20-21 (C). For the hearts of the 3 groups (n = 9-10) together (n = 28) the values of the PGI_2 release were plotted against the corresponding values of $\dot{Q}_{cor}$.

In Fig. 5.3 the individual values of the PGI_2 release from the placebo hearts are plotted against the corresponding values of $\dot{Q}_{cor}$. For all the hearts of the placebo groups together $\dot{Q}_{cor}$ is not systematically related to the PGI_2 release. However, for the (30 HCO + 5 SSO) group at t = 2 and 21 min the relationship between PGI_2 release and $\dot{Q}_{cor}$ was found to be systematic (Table 5.5).

TABLE 5.5 Correlation coefficients of PGI_2 release versus coronary flow rate at 3 perfusion times. Langendorff-perfused hearts (n = 9 - 10) of rats fed various dietary fats for 4 weeks.

Perfusion time/min	Dietary group		
	35 SSO	30 HCO +5 SSO	35 L
2 - 3	0.30	0.84**	–0.27
10 - 11	0.28	–0.27	0.40
20 - 21	0.04	0.75*	0.30

35 SSO: 35 en% sunflowerseed oil, etc. (cf. Table 2.1).
* $P_2 < 0.05$; ** $P_2 < 0.01$.

In Addendum VII (Fig. 2) it was found that when rats were fed Muracon® (cf.2.3.2) perfusion with about 1 nmol indomethacin per ml decreased $\dot{Q}_{cor}$, with about 3 nmol ml^{-1} there was no effect, while perfusion with about 5.5 nmol indomethacin per ml increased $\dot{Q}_{cor}$. This can be explained by the regulation of $\dot{Q}_{cor}$ by c-AMP [22, 36], the influence of PGs on the c-AMP level [22] and the difference in the sensitivities of cyclooxygenase and c-AMP phosphodiesterase for inhibition by indomethacin [15]. When at low concentrations of indomethacin PG synthesis is inhibited, but not the phosphodiesterase, c-AMP will decrease and consequently also $\dot{Q}_{cor}$. When at higher concentrations of indomethacin the phosphodiesterase is also inhibited, c-AMP will rise and consequently also $\dot{Q}_{cor}$.
In the experiment shown in Fig. 5.2 A, no effect on $\dot{Q}_{cor}$ was found at about 1 nmol indomethacin per ml. In this experiment a semisynthetic diet containing 35 en% fat was fed whereas in the experiment mentioned above Muracon® was fed. As in both experiments PG synthesis was inhibited at 1 nmol ml^{-1} indomethacin, this might mean that feeding the semisynthetic diets increased the sensitivity of the c-AMP phosphodiesterase for inhibition by indomethacin. As shown in Fig. 5.2, the sensitivity of the regulation mechanism of $\dot{Q}_{cor}$ to indomethacin is also increased by pretreatment of rats with ASA.
In the experiment, feeding the dietary fats did not lead to systematic differences in m_h (Table 5.6). It was investigated whether there was a relationship between m_h and PGI_2 release. To this end, the correlation coefficients of m_h versus PGI_2 release at t = 2, 11 and 21 min for each of the 3 dietary groups (placebo) and for the 3 groups together were calculated (Table 5.7). The data in this table show that the PGI_2 release is not related to m_h.

TABLE 5.6 Mean values (± SEM; n = 9-10) of dry heart mass (mg) of various groups.

Dietary fat	Placebo group	ASA group
35 SSO	218 ± 7	217 ± 3
30 HCO + 5 SSO	218 ± 4	223 ± 5
35 L	223 ± 3	203 ± 5

For the groups see legend to Fig. 5.2. ASA, acetylsalicylic acid.

TABLE 5.7 Correlation coefficients of dry heart mass versus PGI_2 release at 3 perfusion times. Langendorff-perfused hearts (n = 9-10) of rats fed various dietary fats for 4 weeks.

Perfusion time/min	Dietary group			Groups together
	35 SSO	30 HCO + 5 SSO	35 L	
2 - 3	0.26	0.78*	0.07	0.27
10 - 11	−0.06	−0.30	−0.08	−0.16
20 - 21	0.15	−0.50	0.20	0.10

35 SSO: 35 en% sunflowerseed oil, etc. (cf. Table 2.1).
*$P_2 < 0.05$.

5.4 RELEASE OF THROMBOXANE FROM THE ISOLATED HEART

TXA_2 is mainly synthesized in blood platelets where it contributes to the functioning of the platelets [16]. However, TXA_2 is not only a strong aggregation-inducing substance but also a potent vasoconstrictor [13, 30, 38] and, therefore, TXA_2 released from platelets will be able to affect coronary vessel tone [13]. Although the isolated hearts were perfused with a buffer solution and consequently TX release from platelets cannot be expected, the results show that besides PGs, TXB_2 is released from the isolated heart, although in much smaller amounts than PGI_2 (Table 5.2). Also other authors have found that TXB_2 is released from various organs and cell types [2, 28, 39], particularly during anaphylaxis TXB_2 is released from the isolated heart [3]. In a number of cases it is unclear whether TX is synthesized in the cells belonging to the organ or in blood platelets stuck to the vessel walls of the organ.
In the effluents from the isolated heart TXB_2 was found both in the interstitial and venous effluent (Addendum V, Table 2). Assuming that TX is only released from blood platelets stuck to the vessel wall, then it can be expected that the amount of TXB_2 released into the venous effluent will be several times that of the amount released into the interstitial effluent. The venous effluent

has been in contact with the platelets and will immediately have carried off the TX formed. TXB_2 will then be present in the interstitial effluent due to diffusion and to the fact that the interstitial effluent has first passed the coronary arteries. The TXB_2 concentration in the interstitial effluent will be at most equal to that in the venous effluent. The interstitial effluent is only a few per cents of the venous effluent (cf. Addendum I) and consequently the total amount of TXB_2 released per unit of time into the interstitial effluent will also be a few per cents of the total amount of TXB_2 released into the venous effluent. However, this was not the case. The amounts of TXB_2 released into the venous and interstitial effluents were similar (Addendum V, Table 2). This indicates that TX is also synthesized in heart tissue or in blood cells which have crossed the vessel wall.

TABLE 5.8 Effects of $PGF_{2\alpha}$ on prostaglandin (PG) and thromboxane (TX) release in the Langendorff-perfused rat heart.

Experiment	Perfusion time/min	PG and TX release /pmol min^{-1}			
		PGE_2	$PGF_{2\alpha}$	6-oxo-$PGF_{1\alpha}$	TXB_2
1	10-20 (control)	0.4	0.3	4.8	1.1
	21-31 ($PGF_{2\alpha}$, 2.8 pmol ml^{-1})*	1.0	—	10.0	0.6
2	10-20 (control)	0.4	0.2	4.1	0.6
	21-31 ($PGF_{2\alpha}$, 5.0 pmol ml^{-1})*	1.1	—	8.9	0.4

$PGF_{2\alpha}$ was infused into the perfusion fluid from t = 20-31 min at a rate of 41 (experiment 1) and 82 pmol min^{-1} (experiment 2). Coronary effluent was collected during a period of 10 min, and in pooled effluents from 5 hearts PGs and TXB_2 were determined by gas chromatography. The recovery of $PGF_{2\alpha}$ was about 65%.

*) $PGF_{2\alpha}$ concentration at the start of infusion. Coronary flow rate increased by $PGF_{2\alpha}$ infusion with about 20%.

Mean dry heart masses were 249 (experiment 1) and 227 mg (experiment 2). Perfusion pressure 9.3 kPa.

The next investigation shows that the TX and PG synthesis occur in different cell types, or in one cell type but separated from each other as a result of compartmentalization. In the heart, PG synthesis can be stimulated by a variety of stimuli e.g. by $PGF_{2\alpha}$ [11]. It was investigated whether stimulation of PG synthesis by $PGF_{2\alpha}$ also stimulated the TXB_2 release. To this end, in 2 separate experiments, hearts (n=5) were perfused according to Langendorff (cf. 2.1.2). Coronary effluent was collected before and during perfusion with $PGF_{2\alpha}$. In the pooled effluents, PGs and TXB_2 were determined by gas chromatography (cf. 2.2.1.1). Table 5.8 shows that $PGF_{2\alpha}$ strongly stimulated the

release of PGE_2 and 6-oxo-$PGF_{1\alpha}$, but not that of TXB_2. It is unknown in what way $PGF_{2\alpha}$ stimulates PG synthesis. However, as $PGF_{2\alpha}$ can increase the influx of Ca^{2+} [23] it is likely that PG synthesis is stimulated, because of a stimulation of phospholipase by an influx in Ca^{2+} [25, 33, 35]. Obviously, $PGF_{2\alpha}$ does not stimulate in this case the phospholipase connected with TX synthetase.

5.5 EFFECTS OF EXOGENOUS AND ENDOGENOUS PROSTAGLANDINS ON CORONARY FLOW RATE AND EXTERNAL LEFT VENTRICULAR WORK IN THE ISOLATED RAT HEART

As background information for the explanation of the influences of dietary fat on the isolated rat heart via influences on PG metabolism, the effects of exogenously supplied and endogenously synthesized PGs on $\dot{Q}_{cor}$ and W_{lv} were investigated. In the study in which hearts were perfused with PGs, the effects of the PGs which are released by the isolated rat heart - PGD_2, PGE_2, $PGF_{2\alpha}$ and PGI_2 - were studied. Although PGE_1 is not released by the isolated rat heart, it has often been used in perfusion experiments by other authors. Therefore, the effects of PGE_1 were also studied and compared with the effects of the PGs of the 2-series. Effects of PGs on $\dot{Q}_{cor}$ and cardiac performance have been investigated by others. However, data on the lowest concentrations at which the PGs affect $\dot{Q}_{cor}$ in the Langendorff-perfused heart and data on the effects of the PGs on W_{lv} in the working rat heart were not available for all the PGs. Therefore, in the Langendorff-perfused heart the lowest concentrations of the PGs at which $\dot{Q}_{cor}$ is affected, were determined to compare these concentrations with the concentrations in the coronary effluent.The effects of PGs on contractility were determined in the working heart. To this end, effects of perfusions with PGs and of the inhibition of the PG synthesis on W_{lv}(max) were determined. The investigations have been described in the Addenda VI and VII. Below, the main results together with some additional data are presented and discussed.

Concerning coronary vasodilation, the approximately highest pharmacologically inactive and the approximately lowest active concentration of the PGs are shown in Addendum VI, Table 1. PGE_2 and PGI_2 are the most potent PGs (0.4 - 0.6 pmol ml^{-1}). Under basal conditions, the amount of PGI_2 released by the isolated rat heart is about 8 pmol min^{-1} (Fig. 5.1) resulting in a concentration in the total coronary effluent (at $\dot{Q}_{cor}$ = 12 ml min^{-1}) of about 0.6 pmol min^{-1} which is similar to the concentration which affects $\dot{Q}_{cor}$. It is evident that this concentration is built up during the passage of the coronary effluent through the heart. On the other hand, the local concentration in the vessel wall may be higher than that in the fluid in the vessel lumen. In any case, such a calculation shows that endogenously formed PGs are likely to have some influence on $\dot{Q}_{cor}$.

Concerning contractility, the effect of the PGs on $W_{lv}(max)$ was studied, because the influence of dietary fat on contractility was studied by determining the effect on $W_{lv}(max)$. Addendum VI shows that only PGF_2 increased $W_{lv}(max)$. Although the other PGs increased $\dot{Q}_{cor}$, they did not affect $W_{lv}(max)$. Consequently, an increase in $\dot{Q}_{cor}$ does not affect contractility in the working rat heart. That $PGF_{2\alpha}$ increased contractility has also been found by Januari and Schottelius [23]. These authors found that the effect of $PGF_{2\alpha}$ resembles that of the catecholamines. The $PGF_{2\alpha}$ concentration which gave a distinct increase in $W_{lv}(max)$ was found to be 9 pmol ml^{-1}. The amount of $PGF_{2\alpha}$ released into the interstitial effluent (the fluid in contact with the muscle cells) was found to be 0.2 pmol min^{-1} (calculated from data in Addendum V). The flow rate of the interstitial effluent was about 0.25 ml min^{-1}. Consequently, the PGI_2 concentration in the interstitial effluent was about 0.8 pmol ml^{-1} which is rather low compared with the 9 pmol ml^{-1} needed for a distinct effect on contractility. The recovery of $PGF_{2\alpha}$ in the interstitial effluent was not investigated. Hence that the concentration of $PGF_{2\alpha}$ in the interstitial fluid during perfusion with $PGF_{2\alpha}$ is not known. However, it was found that the total $PGF_{2\alpha}$ recovery from the heart during perfusion with a concentration of 5 pmol ml^{-1} was about 65% (cf. 5.4).

To investigate whether endogenously formed PGs affect $\dot{Q}_{cor}$ and W_{lv} in the isolated rat heart (Addendum VII), PG synthesis was completely inhibited by intraperitoneal injection of 83 μmol ASA per kg body mass, 1.5 h before the isolation of the heart or by perfusion with indomethacin (1-15 nmol ml^{-1}). PG synthesis at the beginning of perfusion could also be completely inhibited by intraperitoneal injection of indomethacin (28 μmol per kg body mass), 1.5 h before isolation of the heart. In this latter case, the inhibition of PG synthesis from endogenous arachidonic acid disappeared after 20-25 min of perfusion. Inhibition of PG synthesis was tested by determining the PG release during perfusion with arachidonic acid or by determining PG release immediately after the start of perfusion. In this latter case, PG release from control hearts is high (Fig. 5.1). Inhibition of PG synthesis by intraperitoneal injection of ASA or indomethacin or by perfusion with indomethacin decreased $\dot{Q}_{cor}$ (Addendum VII) but did not affect $W_{lv}(max)$ (Tables 5.9 and 5.10). Thus it is shown that endogenously formed PGs affect $\dot{Q}_{cor}$ in the Langendorff-perfused heart, but not contractility in the working rat heart.

TABLE 5.9 Effects of indomethacin on external left ventricular work (W_{lv}) and coronary flow rate ($\dot{Q}_{cor}$) in the working rat heart.

Experiment		W_{lv}/J min^{-1}	$\dot{Q}_{cor}$/ml min^{-1}
1 (n=6)	Control	1.34 ± 0.04	23.3 ± 0.9
	Indomethacin (1.1 nmol ml^{-1})	1.34 ± 0.04	23.1 ± 0.9
	Control	1.32 ± 0.04	23.1 ± 0.9
2 (n=4)	Control	1.35 ± 0.05	21.0 ± 1.6[1])
	Indomethacin (8.3 nmol ml^{-1})	1.33 ± 0.05	22.1 ± 1.7
	Indomethacin (15.5 nmol ml^{-1})	1.33 ± 0.05	23.8 ± 1.4[2])
	Control	1.28 ± 0.05	22.4 ± 0.9[3])

Mean values ± SEM. A solution of indomethacin (cf. Addendum VII) was infused into the perfusion fluid upstream from the left atrium, resulting in concentrations indicated in the table. In both experiments, W_{lv} and $\dot{Q}_{cor}$ were determined just before (control), 6 min after the start (indomethacin) and 10 min after the stop (control) of the perfusion with indomethacin. In experiment 2, the indomethacin concentration was increased after the determination of W_{lv} and $\dot{Q}_{cor}$ and 3 min later W_{lv} and $\dot{Q}_{cor}$ were determined again. W_{lv} and $\dot{Q}_{cor}$ were determined at a left ventricular filling pressure of 1.33 kPa.
[1]) versus [2]), and [2]) versus [3]): $P_z < 0.05$.

TABLE 5.10 Effect of acetylsalicylic acid (ASA) on external left ventricular work (W_{lv})and coronary flow rate($\dot{Q}_{cor}$)in the working rat heart.

P_{lvf}[1]) /kPa	ASA group		Control group	
	W_{lv}/J min^{-1}	$\dot{Q}_{cor}$/ml min^{-1}	W_{lv}/J min^{-1}	$\dot{Q}_{cor}$/ml min^{-1}
0.79	1.26 ± 0.04	24.1 ± 1.1	1.24 ± 0.04	24.7 ± 1.0
1.06	1.35 ± 0.04	25.4 ± 0.9	1.34 ± 0.04	25.2 ± 1.0
1.33	1.35 ± 0.04	25.4 ± 0.9	1.35 ± 0.04	25.6 ± 1.1
1.46	1.35 ± 0.04	25.2 ± 0.9	1.37 ± 0.04	26.0 ± 1.1
1.60	1.36 ± 0.04	25.2 ± 0.9	1.37 ± 0.04	26.5 ± 1.1

Mean values ± SEM; n = 6.
[1]) Left ventricular filling pressure.
A solution of ASA (83 μmol kg^{-1}) or saline (control) was injected intraperitoneally, 1.5 h before the isolation of the heart. PG synthesis in the ASA group was completely inhibited which was tested after the determinations of W_{lv} and $\dot{Q}_{cor}$ by perfusion with arachidonic acid (about 0.4 nmol ml^{-1}). Mean dry heart masses: ASA group 217 ± 8 mg; control group 219 ± 6 mg.

5.6 SUMMARY

The prostaglandins (PG) PGI_2 (about 70%), PGE_2 (15%), PGD_2 (5%), $PGF_{2\alpha}$ (5%) and thromboxane (TX; 5%) are released from the isolated, perfused rat and rabbit heart; the relative amounts are given in brackets. It has been discussed that the coronary vessel wall is a likely place for that release of PGs

and TX. The isolation of the heart stimulated PG release. Two to 3 min after the start of the perfusion, PGI_2 release from the isolated rat heart was 22-30 pmol min^{-1} and decreased to about 8 pmol min^{-1} at t = 20-21 min. Under control conditions, the PG release from the Langendorff-perfused and working rat heart were very similar.

After feeding 35 en% sunflowerseed oil (SSO) in comparison with feeding lard (L) or hydrogenated coconut oil (HCO) to rats for 4 weeks, PGI_2 release from the isolated heart immediately after the start of perfusion was somewhat higher and under control conditions somewhat lower; the latter was also found in the isolated, perfused rat aorta, and in the isolated rabbit heart under control conditions or immediately after an anoxic period. However, the differences were not systematic. The TX release from the isolated rat and rabbit heart was not appreciably influenced by the dietary fats either.

In the isolated rat heart PGE_1, PGD_2, $PGF_{2\alpha}$ and PGI_2 dilated the coronary vessels; PGE_2 and PGI_2 most potently, PGE_1 decreased and $PGF_{2\alpha}$ increased contractility; the other PGs were without an appreciable effect. Inhibition of PG synthesis in various ways decreased coronary flow rate ($\dot{Q}_{cor}$), but had no effect on maximum external left ventricular work. After feeding SSO, L or HCO the time courses of $\dot{Q}_{cor}$ from the onset of perfusion were different, but they were very similar after inhibition of PG synthesis.

It is tentatively concluded that the differences in $\dot{Q}_{cor}$ in the Langendorff-perfused heart of rats fed various fats are caused by differences in PGI_2 release immediately after the start of perfusion. However, it has not been investigated whether the differences in $\dot{Q}_{cor}$ are the result of differences in sensitivity of the coronary vessels to PGs. Most probably, PG synthesis in the heart is not involved in the increase in contractility caused by linoleic acid-rich diets.

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6 POSSIBLE ROLE OF THE Na^+,K^+-ATPASE IN THE DIETARY LINOLEIC ACID-INDUCED INCREASE IN CARDIAC CONTRACTILITY

6.1 INTRODUCTION

Chapter 4 shows that feeding linoleic acid-rich diets in comparison with linoleic acid-poor diets increase the myocardial contractility in the working rat heart. The previous chapter shows that in the isolated rat heart prostaglandins are, most probably, not involved in the regulation of myocardial contractility. Therefore, the effect of linoleic acid-rich diets on myocardial contractility has been studied by a different approach.

It is well known that the activity of membrane-bound enzymes and the ligand-receptor-effector interaction in the case of hormones, neurotransmitters or prostaglandins are influenced by the phospholipid classes in the membrane and by the membrane fluidity [19]. Diets containing increasing amounts of linoleic acid have not been found to affect the relative amounts of the phospholipid classes in the rat heart [6], but it is possible that, as a result of the effect of the dietary fats on the fatty acid composition of the phospholipids (cf. 1.2.5 and 4.1.10), the activities of membrane-bound enzymes are affected. For instance, Farias and coworkers [8] found that dietary fat can affect the activity of the myocardial Na^+,K^+-ATPase. This enzyme is very important for the function of the muscle cell. The Na^+,K^+-ATPase influences the intracellular Na^+ concentration and consequently the intracellular Ca^{2+} concentration, so that heart contractility may be affected [3, 14-17, 20].

6.2 EFFECT OF DIETARY FAT ON MYOCARDIAL NA^+K^+-ATPASE

To test whether feeding diets containing sunflowerseed oil (SSO), lard (L) or hydrogenated coconut oil (HCO) affect the Na^+,K^+-ATPase activity, rats were fed experimental diets (cf. 2.3.2) containing 35 en% SSO, 33 en% L + 2 en% SSO or 30 en% HCO + 5 en% SSO for 4 weeks after which the rate of the myocardial $^{86}Rb^+$ uptake was determined (cf. 2.2.5). The $^{86}Rb^+$ uptake is a measure of the Na^+,K^+-ATPase activity. From Table 6.1 it can be seen that feeding the SSO-rich diets, compared to the L or HCO diets, tends to decrease the Na^+,K^+-ATPase activity.

TABLE 6.1 Ouabain-sensitive uptake of $^{86}Rb^+$ in heart tissue of rats fed various dietary fats for 4 weeks.

Experiment	Dietary group	$^{86}Rb^+$ uptake /nmol per 100 mg wet mass per 30 min
1	35 SSO	2.80 ± 0.27
	33 L + 2 SSO	3.53 ± 0.40
	30 HCO + 5 SSO	3.27 ± 0.20
2	35 SSO	2.85 ± 0.20
	33 L + 2 SSO	3.18 ± 0.30

35 SSO: 35 en% sunflowerseed oil, etc. (Table 2.1).
Mean values ± SEM; n = 10.

The values of the ouabain-sensitive $^{86}Rb^+$ uptake in the heart slices show that the uptake per 100 mg wet mass is rather low, and the variation in the results great. In a preliminary study it was found that the ouabain-sensitive $^{86}Rb^+$ uptake per unit of mass of atrial tissue was about 8 times that of ventricular slices. A reason for this is that atrial tissue is much less damaged by the preparation than ventricular slices. Therefore, by using atrial tissue the variation in the values of the $^{86}Rb^+$ uptake may be lowered. A dietary study, however, using atrial tissue has not been performed.
The difference in the Na^+,K^+-ATPase activities might have been caused by the effect of the dietary fats on the membrane properties as hypothesized by Farias *et al.* [8]. However, the myocardial Na^+,K^+-ATPase is also strongly affected by T_3 and T_4 [5, 13], and therefore, the involvement of thyroid hormone is considered in the next section.

6.3 ROLE OF THYROID HORMONE

Since it has been found that dietary fat can affect the activity of the thyroid gland [9, 18, 24] and may interfere with the binding of thyroxine to plasma albumin [11, 23], dietary fat may influence myocardial contractility via the plasma T_3 and T_4 levels. To test this possibility, the effect of lowering plasma thyroxine on the increase in contractility induced by feeding SSO was investigated. Rats were fed experimental diets (cf. 2.3.2) containing 35 en% SSO or 33 en% L + 2 en% SSO for 5 weeks. In half the number of rats of both dietary groups, plasma thyroxine was lowered by methimazole[1]) [12], which was supplied in the drinking water (0.5 mg l^{-1}) during the last 2 weeks of the experiment. The other half was used as control. Within a few days, methimazole decreased the water consumption from about 28 ml (control rats) to about 13 ml per day per rat, by which the methimazole uptake became about 6.5 mg per day.

[1]) Methimazole (1-methyl-2-mercaptoimidazole) was obtained from Nogepha, Alkmaar, The Netherlands.

TABLE 6.2 Body mass (m_b) and dry heart mass (m_h) of rats after various dietary regimens and treatment with methimazole.

Dietary group	m_b/g			m_h/mg
	Experimental feeding period/week			
	0	3	5	
35 SSO	186 ± 3	274 ± 6	314 ± 7	218 ± 7
35 SSO + methimazole	186 ± 3	278 ± 6	264 ± 6	160 ± 6
33 L + 2 SSO	186 ± 3	274 ± 6	302 ± 6	212 ± 7
33 L + 2 SSO + methimazole	186 ± 3	279 ± 6	263 ± 7	165 ± 6

35 SSO: 35 en% sunflowerseed oil, etc. (Table 2.1). The diets were fed for 5 weeks. Methimazole was present in the drinking water during the last 2 weeks. Mean values ± SEM; n=10.

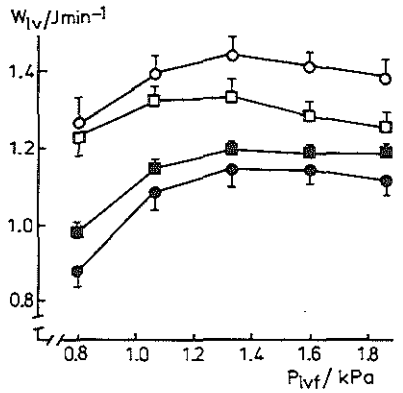

Fig. 6.1 Relationship between left ventricular filling pressure (P_{lvf}) and external left ventricular work (W_{lv}) of working hearts of rats fed 35 SSO (o———o), 35 SSO + methimazole (●———●), 33 L + 2 SSO (□———□), or 33 L + 2 SSO + methimazole (■———■). For the details of the dietary groups see legend to Table 6.2. The decrease in the mean values of W_{lv} by the treatment with methimazole was mainly caused by the decrease in heart mass. Mean values ± SEM; n = 7-8.

Feeding the 2 dietary fats did not lead to differences in mean body and dry heart mass (m_h), but methimazole decreased both parameters (Table 6.2). In the isolated, working heart the relationship between left ventricular filling pressure (P_{lvf}) and external left ventricular work (W_{lv}) was determined. Fig. 6.1 shows that feeding 35 en% SSO increased the contractility with regard to feeding 33 en% L + 2 en% SSO, and that by the methimazole treatment the in-

crease in contractility was prevented. W_{lv} in the methimazole groups is lower than in the control groups. This is mainly caused by the decrease in m_h by methimazole and the dependence of W_{lv} on m_h (cf. chapter 3). The influences of both m_h and methimazole on the maximal value of W_{lv} (W_{lv}(max)) were evaluated statistically by analysis of covariance. This analysis showed that W_{lv}(max) is related to m_h ($P < 0.001$) (Fig. 6.2) and that the regression coefficients of the relationship between m_h and W_{lv}(max) within each group did not differ systematically. Therefore, the pooled regression coefficient was used to adjust the values of W_{lv}(max) to $m_h = 220$ mg. The adjusted values of W_{lv} (max) are listed in Table 6.3. The statistical analysis further showed that there was a statistically significant ($P < 0.05$) fat-methimazole interaction which means that by the treatment with methimazole the difference in W_{lv} (max) between the SSO and L group had disappeared.

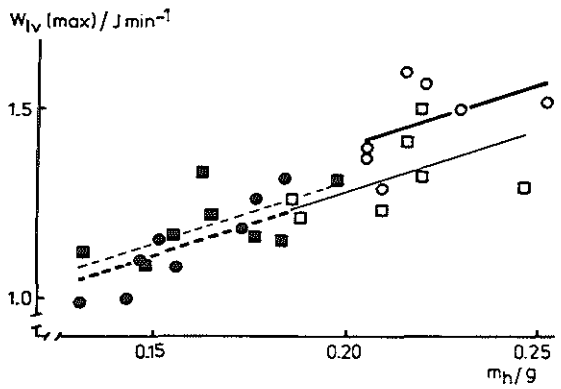

Fig. 6.2 Relationship between dry heart mass (m_h) and maximal external left ventricular work (W_{lv}(max)) in 4 groups of hearts of rats after various dietary regimens and treatment with methimazole.
For details of the dietary groups see legend to Table 6.2. The relationship between m_h and W_{lv}(max) was evaluated statistically by analysis of covariance ($P < 0.001$). Since in the analysis of covariance it was found that the regression coefficients did not differ significantly ($P = 0.29$), the pooled regression coefficient (3.88) was used for the drawing of the regression lines of the relationship between m_h and W_{lv} (max) within each group. ○,———: 35 SSO; ●,— — —; 35 SSO + methimazole; □,———: 33 L + 2 SSO; ■,— — —: 33 L + 2 SSO + methimazole.

TABLE 6.3 Parameters determined in the isolated, working rat heart after various dietary regimens and treatment with methimazole.

Dietary group	Coronary flow rate /ml min^{-1}	Left ventricular output /ml min^{-1}	Mean aortic pressure /kPa	Maximal external left ventricular work/J min^{-1}	
				determined	adjusted[1])
35 SSO (n=7)	27.7 ± 1.1	109 ± 4	14.6 ± 0.1	1.46 ± 0.05	1.46 ± 0.037
35 SSO + methimazole (n=8)	23.4 ± 0.5	94 ± 2	13.6 ± 0.1	1.15 ± 0.01	1.34 ± 0.023
33 L + 2 SSO (n=7)	26.1 ± 0.8	102 ± 3	14.2 ± 0.1	1.32 ± 0.05	1.34 ± 0.037
33 L + 2 SSO + methimazole (n=8)	24.4 ± 0.7	98 ± 3	13.6 ± 0.1	1.20 ± 0.05	1.37 ± 0.027

Mean values ± SEM obtained at left ventricular filling pressure = 1.33 kPa. For details of the dietary groups see legend to Table 6.2. The differences in the mean values of the parameters between the methimazole and control groups were mainly caused by the decrease in heart mass by the treatment with methimazole.

[1]) Adjusted to dry heart mass = 220 mg by analysis of covariance. A statistically significant ($P<0.05$) interaction between methimazole and fat was found.

Neither the dietary fats nor methimazole affected the value of P_{lvf} (1.33 kPa) at which W_{lv} (max) was obtained (Fig. 6.1). The mean values of coronary flow rate, left ventricular output, mean aortic pressure and W_{lv}(max) at P_{lvf} = 1.33 kPa are also tabulated in Table 6.3.

It has been found by others that methimazole has a number of important effects. Besides the decrease in plasma thyroxine, it increases plasma cholesterol and decreases liver lipase activity [12]. No data are available about the effects on the heart of the latter two findings. The decrease in plasma thyroxine can also affect the fatty acid desaturation [4, 7]. Peifer [21] found that in hyperthyroid rats the (n-6)/(n-3) ratio of the fatty acids in the myocardial phospholipids was decreased. However, in our experiment the fatty acid composition of the phospholipids was affected only slightly by lowering thyroid hormone (Table 6.4), and it is unlikely that the small changes found can explain the disappearance of the difference in contractility between the SSO and L group. The small changes found in the fatty acid composition of the myocardial phospholipids, an increase in the (n-3) and a decrease in the (n-6) fatty acids, evoked by methimazole, are in accordance with the results of Peifer [21].

TABLE 6.4 Fatty acid composition of the myocardial phospholipids of rats after various dietary regimens and treatment with methimazole.

Fatty acid	Dietary group			
	35 SSO	35 SSO + methimazole	33 L + 2 SSO	33 L + 2 SSO + methimazole
C 16:0	8.1	9.2	10.9	11.1
C 18:0	22.6	21.9	23.3	20.9
C 18:1 (n-9)	5.5	5.4	7.5	7.5
C 18:2 (n-6)	22.0	21.3	10.4	12.2
C 20:0	0.3	0.4	0.2	0.3
C 20:1 (n-9)	0.3	0.2	0.3	0.3
C 20:4 (n-6)	23.5	21.8	23.9	22.4
C 20:5 (n-3)	0.5	0.4	0.7	0.8
C 22:4 (n-6)	2.4	1.9	1.1	1.0
C 22:5 (n-6)	3.5	2.9	1.5	1.1
C 22:5 (n-3)	0.6	0.9	2.3	2.1
C 22:6 (n-3)	5.5	7.6	11.9	12.7

For details of the dietary groups see the legend to Table 6.2. For the determination of the fatty acid composition of the myocardial phospholipids see section 2.2.4. Pooled samples of 7 hearts.

It has been found that a decrease in thyroid hormone leads to a decrease in myosin ATPase activity [22]. Assuming that feeding linoleic acid-rich diets may lead to a decrease in thyroid hormone, then it is not likely that this effect

will be great since after feeding SSO or L for 4 weeks the myocardial myosin ATPase activities were very similar (Addendum IV, Results section).

6.4 DISCUSSION

It is well known that inhibition of the Na^+,K^+-ATPase activity (e.g. by ouabain) increases myocardial contractility. Therefore, the increase in the contractility by feeding linoleic acid-rich diets may be explained by the decrease observed in the Na^+,K^+-ATPase activity. This decrease might be caused by an effect of the dietary fats on membrane composition or on circulating T_3 and T_4 levels. That thyroid hormone could be involved is not only supported by the finding that treatment with methimazole eliminated the difference in the contractility between the SSO- and L-fed groups, but also by a number of literature data. On the one hand, Alam and Alam [1] showed that EFA deficiency increased Na^+,K^+-ATPase activity. On the other hand, Gambal and Quackenbush [9] found that EFA deficiency increased the amount of plasma bound iodine, and they concluded that the secretion of thyroidal iodine is dependent on essential fatty acids. This is supported by recent results of Boeynaems and coworkers [2], who found that arachidonic acid depressed the thyrotropin stimulated secretion of thyroid hormone (which was not mediated by prostaglandins).

6.5 SUMMARY

Feeding to rats diet containing sunflowerseed oil in comparison with lard or hydrogenated coconut oil for about 4 weeks tended to decrease myocardial Na^+,K^+-ATPase activity, but the myocardial myosin ATPase activities were very similar. Treatment of rats fed sunflowerseed oil or lard with methimazole made disappear the difference in the maximal external left ventricular work determined in their working hearts. It might be possible that the increase in maximal left ventricular work as observed after feeding linoleic acid-rich diets is caused by a small decrease in plasma T_3 and T_4 and consequently in myocardial Na^+,K^+-ATPase activity.

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7 GENERAL DISCUSSION

7.1 INTRODUCTION

The object of this study was to investigate whether the type of dietary fat fed to rats for a relatively short period, affects coronary flow rate and cardiac performance in their isolated, perfused hearts. From literature data it was already evident that dietary fat can affect heart function (section 1.2). However, the dietary regimens generally used were rather extreme, e.g. long experimental feeding periods were applied and the effects of dietary fats with particular properties like fish oils and rapeseed oil, and of essential fatty acid (EFA) deficiency were investigated. In the present study extreme experimental feeding conditions have been avoided. Usually, the amount of fat in the experimental diets was 35 per cent of the digestible energy (35 en%); EFA deficiency was avoided by feeding always at least 3 en% linoleic acid, and the experimental feeding times were short, up to 6 weeks.

The rat was chosen as animal model. The rat shows a number of differences in lipid metabolism as compared with man. For instance, the rat does not possess a gall-bladder as a result of which diets containing very high amounts of fat (>50 en%) may not be tolerated well. For this reason we usually fed diets containing 35 en% fat. Another difference with man is the very low amount of plasma low density lipoprotein. A third difference is that the main cholesteryl ester in the rat is that of arachidonic acid, while in man it is that of linoleic acid. Nevertheless, the rat was chosen because of the existing experience with this model.

7.2 LINOLEIC ACID AS THE ACTIVE DIETARY AGENT

Feeding rats linoleic acid-rich diets, in comparison with linoleic acid-low diets, increased coronary flow rate ($\dot{Q}_{cor}$) and maximal external left ventricular work ($W_{lv}(max)$) in their isolated hearts. That linoleic acid is the dietary factor which increases these heart parameters can be concluded from the following findings. Only after feeding sunflowerseed oil (about 70% linoleic acid) were $\dot{Q}_{cor}$ and $W_{lv}(max)$ systematically increased with respect to the values obtained after feeding linoleic acid-low fats (chapter 4); a positive, linear relationship was found between the dietary linoleic acid / saturated fatty

acids ratio and W_{lv}(max) (Fig. 4.5), and feeding the artificial fat trilinoleoylglycerol (98% linoleic acid), in comparison with tripalmitoylglycerol (98% palmitic acid), led to a systematically higher value of W_{lv}(max) (Fig. 4.4).

7.3 EFFECT OF LINOLEIC ACID-RICH DIETS ON CORONARY FLOW RATE

$\dot{Q}_{cor}$ in the isolated heart perfused with Krebs-Henseleit buffer (KH buffer) is high when compared with $\dot{Q}_{cor}$ in the heart in the intact animal or in isolated hearts perfused with blood or KH buffer to which erythrocytes have been added (section 2.4). Despite this, the Langendorff heart perfused with KH buffer may provide information on the possible influence of dietary fat on coronary vascular resistance.

In most experiments, $\dot{Q}_{cor}$ was determined after an equilibrium period of 15 min, for 5 to 10 min and the average value of $\dot{Q}_{cor}$ during this period was used to compare the effects of the various dietary fats on $\dot{Q}_{cor}$. Under these conditions, feeding sunflowerseed oil in comparison with hydrogenated coconut oil or lard increased $\dot{Q}_{cor}$ (Addenda III and IV). In one experiment the influence of feeding these 3 fats on the time course of $\dot{Q}_{cor}$ from the onset of perfusion was determined (Fig. 5.2 A). Although the differences between the time courses going with the 3 fats were not statistically significant, Fig. 5.2 B shows that the differences disappeared as a result of the inhibition of prostaglandin (PG) synthesis.

As in the isolated rat heart $\dot{Q}_{cor}$ is affected by PG synthesis (Addendum VII) and as feeding linoleic acid-rich diets stimulates PG synthesis *in vivo* [12, 17, 24, 25, 31, 38], the increase in $\dot{Q}_{cor}$ found after feeding linoleic acid-rich diets may have been caused by an increase in the PG synthesis in the isolated heart. Although after feeding linoleic acid-rich diets, in comparison with linoleic acid-low diets, the basal PG release (from 15 min after the start of perfusion) tended to be somewhat lower (Addendum V), by which it is unlikely that the basal PG release is involved in the increase observed in $\dot{Q}_{cor}$, the effect of the inhibition of the PG synthesis on the time course of $\dot{Q}_{cor}$ (Fig. 5.2) shows that PGs could still be involved in the causation of the differences in $\dot{Q}_{cor}$ obtained after feeding the various dietary fats. A possibility is the existence of differences in PG synthesis at the beginning of the perfusion of the heart. During the isolation of the heart a short ischemic period occurs, as a result of which PG synthesis is stimulated. Several authors have found that feeding linoleic acid-rich diets, in comparison with linoleic acid-low diets, increases the stimulated PG release [5, 20]. We also found that the stimulated PG release (immediately after the isolation of the heart) was increased to some extent by the linoleic acid-rich diet (Fig. 5.1). Therefore, the differences in $\dot{Q}_{cor}$

found after feeding the various dietary fats might have been caused by an influence on the PG synthesis immediately after the isolation of the heart.
A number of studies show that the sensitivity of ligand-receptor-effector mechanisms for PGs can be affected by the membrane fluidity and the type of dietary fat [33, 34]. Therefore, another explanation might be that the sensitivity of coronary vascular smooth muscle to PGs is affected by the type of dietary fat. However, no investigations were performed on this point.

7.4 EFFECT OF LINOLEIC ACID-RICH DIETS ON CARDIAC PERFORMANCE

7.4.1 Methodological considerations

The effect of the dietary fats on cardiac performance has been investigated by the determination of the left ventricular function curve (Figs. 2.9 and 2.10) in the isolated, working rat heart. The peak value of the function curve (W_{lv}(max)) is used as a parameter of cardiac performance (section 2.5). The W_{lv}(max) values as found in our study are similar or higher than comparable values of the external left ventricular work (W_{lv}) obtained in the intact animal or under other experimental conditions by other authors (Table 2.2). Therefore, the isolated, working heart appears to be a reliable preparation to investigate cardiac performance. Moreover, it is a relatively simple technique.
Several other methods are used to study cardiac performance, but all these methods have one or more disadvantages. W_{lv} can also be determined in the rat heart in the intact animal (section 2.4) or in the heart-lung preparation. In the former preparation it is not possible to determine a function curve as the increase in heart work has to be obtained by increasing blood volume by adding a dextran solution or by increasing left ventricular afterloading (e.g. by angiotensin II). In the latter preparation, aggregation of blood platelets occurs as a result of which the performance of the heart diminishes. Cardiac performance can also be studied by using a latex balloon in the left ventricular cavity. However, we found that this method is not suitable for comparative studies for the following reasons. Inserting the balloon in the left ventricle disturbs the heart, the volume of the balloon cannot be determined accurately at every moment, and the end-diastolic pressure in the balloon depends on the wall properties of the balloon which are variable. For measurements of the contractile force, the Langendorff-perfused heart with a load attached to the apex is used. The load deforms the ventricle as a result of which the determination of contractility is inaccurate and not very reliable [3, 9]. Moreover, we found that the amount of fluid in the wall (interstitial and vascular fluid; edema formation) affects the displacement of the apex. Determination of myocardial contractility can be performed accurately in the papillary muscle preparation. However, the oxygenation of the inner muscle

fibres is possibly inadequate [11]. Particularly, when there are differences in oxygen consumption or oxygen diffusion between groups of papillary muscles, this will complicate the interpretation of the results.

7.4.2 Explanation of the increase in myocardial contractility by linoleic acid-rich diets

Feeding linoleic acid-rich diets in comparison with linoleic acid-low diets caused an upward shift of the left ventricular function curve (Addenda III and IV; Fig. 4.4); the value of the left ventricular filling pressure at which W_{lv}(max) was reached, was not affected by the type of the dietary fat. On the basis of the arguments outlined in section 2.5, therefore, it can be concluded that linoleic acid-rich diets increase myocardial contractility.

A number of investigations have been performed to explain this phenomenon. It has been found by others that training of rats leads to a similar shift in the left ventricular function curve [26]. This shift was explained by an increase in the myosin ATPase activity [1]. However, after feeding sunflowerseed oil or lard, the myosin ATPase activities were very similar (Addendum IV). PGs are synthesized in the isolated rat heart (Addendum II). A number of PGs have a positive inotropic effect and $PGF_{2\alpha}$ increases W_{lv}(max) in the working rat heart (Addendum VI). However, inhibition of the PG synthesis did not affect W_{lv}(max) (Tables 5.9 and 5.10) which rules out the possible involvement of the cardiac PG synthesis. Dietary fat can affect the plasma T_3 and T_4 levels or the membrane fluidity (chapter 6) which in turn can affect the myocardial Na^+,K^+-ATPase activity, and consequently contractility. Feeding linoleic acid-rich diets decreased the myocardial Na^+,K^+-ATPase activity indeed, although not systematically, which might explain the increase in contractility. In chapter 6, the role of thyroid hormone has been discussed; feeding a linoleic acid-rich diet might lead to a small decrease in plasma T_3 and T_4 and subsequently to the observed decrease in myocardial Na^+,K^+-ATPase activity.

Myocardial contractility may also be affected by effects of dietary fat on membrane fluidity and membrane composition. For instance, dietary fat affects the fatty acid composition of membrane phospholipids (sections 1.2.5 and 4.1.10). These latters play an important part in the binding of Ca^{2+} to the membrane [27]. As it had been found that membrane-bound Ca^{2+} influences contractility [13, 28, 32], it might be that, as a result of changes in the fatty acid composition of the phospholipids evoked by a diet rich in linoleic acid, the affinity of the membrane for Ca^{2+}, and consequently the Ca^{2+} pool available for initiating contraction, is increased.

Another possible explanation is, like the former one, also based on literature data. The rate of the endogenous fatty acid supply for oxidation can affect myocardial contractile force [36]. It has also been found that the oxidation

rate of linoleic acid is somewhat faster than that of other long-chain fatty acids (section 1.3.4) [6, 8, 14, 21] and that feeding linoleic acid-rich diets may increase the rate of myocardial substrate oxidation (section 1.2.4.5). These phenomena might contribute to the increase of contractility after feeding the linoleic acid-rich diets. However, an increase in the rate of (endogenous) fatty acid oxidation is not to be expected as an increase in the rate of myocardial oxygen consumption was not found (Addendum III and IV). Neither did we find differences in the rates of oxidation and phosphorylation in mitochondria, isolated from hearts of rats fed sunflowerseed oil or lard for 6 weeks (Addendum IV). Therefore, it is not likely that the increase in contractility can be explained on the bests of the above mentioned literature data.

7.5 EFFECT OF LINOLEIC ACID-RICH DIETS ON PROSTAGLANDIN RELEASE

After feeding linoleic acid-rich diets (24 en% linoleic acid) in comparison with linoleic acid-low diets (3 en% linoleic acid) a tendency has been found for a somewhat lower PG release from the isolated heart during control perfusion conditions (basal PG release). To understand this finding, an insight in the causation of the basal PG release is necessary. Generally, PGs are released as a result of a specific, hormonal or a non-specific, traumatic stimulation of a phospholipase (A_2 or C). Under control perfusion conditions a hormone (from endogenous origin) - mediated PG release from the isolated heart has not been demonstrated. The continuous, basal PG release found is large enough to affect $\dot{Q}_{cor}$ (section 5.5; Addendum VII), but in the intact animal a basal PG release from the rat and rabbit heart, if present, is so low that the coronary blood flow is not affected by inhibiting PG synthesis [2, 7]. Therefore, it is likely that the PG synthesis in the isolated heart is induced by the perfusion conditions (a traumatic condition), and that the somewhat lower basal PG release found after feeding linoleic acid-rich diets might indicate that the heart became somewhat less vulnerable to these conditions.

Immediately after the isolation of the heart (stimulation of PG synthesis by ischemia), the PGI_2 release from hearts of rats fed a linoleic acid-rich diet was somewhat higher than from hearts of rats fed a linoleic acid-low diet. However, after an adaptation period of about 15 min (control condition), there was no appreciable difference (Fig. 5.1). This is in accordance with literature data. Feeding linoleic acid-rich diets increases the (hormone -) stimulated PG synthesis [5, 20], but not the basal PG synthesis [20]. As discussed above, the basal PG release from the isolated rat heart is most likely caused by the non-specific stimulation of PG synthesis due to the experimental conditions. In this case, arachidonic acid for PG synthesis is derived from other pools in addition to the specific pool connected with the phospholipase-cyclooxygenase mechanism [15, 16], and as feeding more than 3 en% linoleic acid hardly af-

fects the amount of arachidonic acid in the cardiac phospholipds (Fig. 4.3; section 1.2.5), an increase in the basal PG release cannot be expected. About the increase in the stimulated PG release only speculations can be made. No information is available as to whether the arachidonic acid content in the specific phospholipid pool (phosphatidyl inositol) involved in the hormone-stimulated PG synthesis, is influenced in another way by the amount of dietary linoleic acid than total cardiac phospholipid (cf. Table 1.3). The amount of arachidonic acid in the triacylglycerols and in the cholesteryl esters in the heart are affected by the amount of dietary linoleic acid [19, 29, 37] and these pools might play a part in the turnover of the phospholipids involved in the stimulated PG synthesis. However, dietary linoleic acid may interfere with PG synthesis in another way than only by affecting the substrate level, since literature data indicate that PG synthesis not only depends on substrate availability [10, 18, 35]. Moreover, several other factors have been described which stimulate or inhibit PG synthesis [4, 22, 23, 30].

7.6 EXTRAPOLATION OF THE RESULTS TO MAN

An increase in coronary flow rate and myocardial contractility as observed in the isolated rat heart after feeding linoleic acid-rich diets, in comparison with linoleic acid-low diets, might be benificial to man in cases of angina pectoris, myocardial infarction and heart failure. For further extrapolation of the outcome of the animal studies to man it is important to know the exact causes of the increases in the coronary flow rate and contractility. In this study only indications as to these causes have been obtained and further investigations seems worthwile.

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SUMMARY

In the isolated perfused rat heart, a study was performed to investigate the effects of the type of dietary fat, and particularly those of the amount of dietary linoleic acid, on coronary flow rate and cardiac performance. The rat was chosen as model, because many data are available about this animal, our experience with this model, and the rat get atherosclerosis only when fed certain diets (which were not used in this study).

Male Wistar rats were fed complete diets containing 23 en% casein and usually 35 en% fat and 42 en% starch. The diets were fed for periods varying from 3 days to 6 weeks. After the experimental feeding periods the rats were 4-6 months old. The fats investigated comprised the natural fats sunflowerseed oil, soyabean oil, olive oil, linseed oil and lard, the hydrogenated fats of soyabean and coconut oil, and the artificial fats trilinoleoylglycerol (TLG; 98% linoleic acid) and tripalmitoylglycerol (TPG; 98% palmitic acid). All diets contained at least 3 en% linoleic acid.

At the end of the experimental feeding period the heart was isolated under anesthesia, perfused with oxygenated Krebs-Henseleit buffer supplemented with 11.1 mmol l^{-1} glucose and 0.5 mmol l^{-1} EDTA, and paced electrically at a frequency of 360 min^{-1}. For the investigation of the effect of the type of dietary fat on coronary flow rate ($\dot{Q}_{cor}$) and cardiac performance the Langendorff heart preparation in which the heart is perfused via the aorta, and the working heart preparation in which the heart is perfused via the left atrium were used. $\dot{Q}_{cor}$ was determined by measuring the amount of coronary effluent per min, and cardiac performance by determining the left ventricular function curve. The latter was determined by measuring external left ventricular work (W_{lv}) at increasing values of left ventricular filling pressure. The latter is representative of left ventricular end-diastolic pressure. The peak value of the function curve is called the maximal value of W_{lv} (W_{lv}(max)) and it is used as an index of myocardial contractility.

In the Langendorff-perfused heart the type of dietary fat affected $\dot{Q}_{cor}$. Generally, feeding sunflowerseed oil in comparison with lard or hydrogenated coconut oil systematically increased $\dot{Q}_{cor}$ when measured after a period of adaptation of the heart to the perfusion circumstances. The increase was

up to 15% and was already found after about 1 week of feeding. When rats were fed mixtures of sunflowerseed oil and hydrogenated coconut oil, $\dot{Q}_{cor}$ increased linearly with the amount of sunflowerseed oil. Also the time course of $\dot{Q}_{cor}$ from the onset of perfusion was affected by the type of dietary fat (see below). In the working heart only feeding sunflowerseed oil in comparison with other fats systematically increased W_{lv}(max), reflecting an increase in contractility. The difference was about 10% and was found after 1 week of feeding sunflowerseed oil and hydrogenated coconut oil or after 4 weeks of feeding sunflowerseed oil and lard.

As sunflowerseed oil is rich in linoleic acid (about 70%) it was investigated whether linoleic acid was the cause of the increase in contractility. To this end, diets containing 20 en% TLG + 5 en% TPG and 5 en% TLG + 20 en% TPG were fed for 4 weeks. After the TLG-rich diets W_{lv} (max) was 11% higher. Moreover, after feeding mixtures of SSO and L a systematic, linear relationship between the dietary linoleic acid / saturated fatty acids ratio and W_{lv}(max) was found.

None of the dietary fats tested affected growth during the experimental feeding period, dry heart mass or the spontaneous frequency of the isolated heart. In the isolated heart, after feeding sunflowerseed oil, lard or hydrogenated coconut oil oxygen consumption per unit of external work, glucose uptake and the release of lactate and long-chain fatty acids were similar. In isolated mitochondria the ADP / O ratio and the rate of ATP synthesis were not affected.

As dietary linoleic acid is the likely causal factor of the increase in $\dot{Q}_{cor}$ and W_{lv}(max), and as linoleic acid is the ultimate precursor of the prostaglandins (PG) of the 1- and 2-series, it was investigated whether the effects found were mediated by an influence on the PG synthesis in the isolated rat heart. To this end some general aspects of PGs in the isolated heart and the effect of the type of dietary fat on PG synthesis were investigated.

Under basal or control conditions, the isolated heart released PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and thromboxane B_2. PGI_2 was determined as 6-oxo-$PGF_{1\alpha}$ release (gas chromatography) or as PGI_2-like activity (inhibition of the ADP-induced platelet aggregation). PGI_2 was the main PG released (about 70%) and the release under control conditions was about 8 pmol min^{-1} (in the range of the heart masses measured, no relationship was found between heart mass and PGI_2 release). Perfusion with PGD_2, PGE_2, PGF_2, $PGF_{2\alpha}$, PGI_2 or PGE_1 increased $\dot{Q}_{cor}$; PGE_2 and PGI_2 were the most potent vasodilators (0.4-0.6 pmol ml^{-1}). In the working heart only $PGF_{2\alpha}$ (9 pmol ml^{-1}) increased W_{lv}(max), whereas PGE_1 (14 pmol ml^{-1}) decreased W_{lv}(max). As a result of the inhibition of PG synthesis in the heart by intraperitoneal injection of acetylsalicylic acid or indomethacin about 1 h before heart isolation, or by perfusion with indo-

methacin, $\dot{Q}_{cor}$ was decreased which indicates that in the isolated heart $\dot{Q}_{cor}$ is affected by the endogenous PG synthesis. Inhibition of the PG synthesis did not have any effect on $W_{lv}(max)$ which shows that $W_{lv}(max)$ is not affected by the endogenous PG synthesis. .

After feeding linoleic acid-rich diets (about 24 en% linoleic acid), in comparison with linoleic acid-low diets (about 3 en% linoleic acid), the PG release from the isolated heart immediately after the start of perfusion tended to be increased, but under control perfusion conditions the PG releases were similar. Feeding such diets resulted in different time courses of $\dot{Q}_{cor}$ from the onset of perfusion. As a result of the inhibition of PG synthesis by intraperitoneal injection of acetylsalicylic acid these differences completely disappeared. Therefore, it may be that the differences in the time courses of $\dot{Q}_{cor}$ were caused by differences in PGI_2 synthesis immediately after the start of the perfusion, although an effect on the sensitivity of the coronary vessels for PGs cannot be excluded.

After feeding sunflowerseed oil, in comparison with lard or hydrogenated coconut oil, the myocardial Na^+, K^+-ATPase activity was somewhat lower (about 10%). By strongly lowering plasma thyroxine, the differences in $W_{lv}(max)$ between the groups fed sunflowerseed oil or lard completely disappeared. Therefore, as thyroxine affects the myocardial Na^+, K^+-ATPase activity, it may be that thyroxine is involved in the increase in contractility evoked by the sunflowerseed oil-rich diets.

It can be concluded that in the isolated rat heart coronary flow rate and contractility increase with the amount of dietary linoleic acid. The effect on coronary flow rate might be caused by an effect on the prostaglandin synthesis at the beginning of the heart perfusion and the effect on contractility by a small decrease in myocardial Na^+,K^+-ATPase activity. For the extrapolation of the findings to man it is necessary to further investigate the causes of these findings.

SAMENVATTING

Om meer inzicht te verkrijgen in de effecten van voedingsvetten op het hart, werd een onderzoek uitgevoerd naar de invloed van verschillende voedingsvetten en in het bijzonder van de hoeveelheid linolzuur in het voedsel, op de doorstroming en arbeid in het geïsoleerde hart van de rat. De rat werd als diermodel gekozen, omdat van dit dier veel gegevens met betrekking tot het hart bekend zijn en gezien onze experimentele ervaring met dit proefdier. Bovendien krijgen ratten alleen bij bepaalde, door ons niet gebruikte diëten atherosclerose, zodat hiermee geen rekening behoefde te worden gehouden.
Mannelijke Wistar ratten kregen gedurende drie dagen tot zes weken experimentele diëten, met als hoofdbestanddelen: 23 en% (% van de verteerbare energie) caseïne, meestal 35 en% vet (waarvan altijd tenminste 3 en% linolzuur) en 42 en% zetmeel. Aan deze diëten werden alle benodigde mineralen en vitaminen in voldoende mate toegevoegd. De volgende voedingsvetten werden onderzocht: zonnebloemolie (SSO), sojaolie, olijfolie, lijnolie, reuzel (L), geharde sojaolie, geharde cocosnootolie (HCO) en één kunstmatig vet met 98% linolzuur (TLG) en één met 98% palmitinezuur (TPG).
Aan het eind van de experimentele voedingsperiode werd het hart onder narcose uit het dier gehaald (geïsoleerd) en werd de aorta verbonden met een vat met een Krebs-Henseleit buffer, voorzien van glucose en zuurstof (perfusievloeistof). De bloedvaten van het hart (coronaire vaten) werden aldus via de aorta doorstroomd (geperfundeerd) met de perfusievloeistof. Deze methode wordt de perfusie volgens Langendorff genoemd. Tijdens deze perfusie verricht het hart geen noemenswaardige uitwendige arbeid. Om een hartpreparaat (het werkende hart) te verkrijgen dat wel uitwendige arbeid verricht, werd ook de linkerboezem met het vat met de perfusievloeistof verbonden. Na een praeperfusie volgens Langendorff werd de toevoer naar de linkerboezem geopend en die naar de aorta gesloten. Aldus werd de linkerkamer gevuld en de vloeistof vervolgens uitgepompt tegen een hydrostatische druk in de aorta van 9,3 kPa (70 mm Hg) (diastolische waarde). De druk, waarmee de kamer werd gevuld (P_{lvf}) was instelbaar (0.5 - 2.0 kPa (4-15 mm Hg)).
De coronaire stroomsterkte ($\dot{Q}_{cor}$) werd bepaald door de vloeistof die uit het hart stroomt per tijdseenheid op te vangen. De door de linkerkamer per mi-

nuut verrichte hoeveelheid uitwendige arbeid (W_{lv}) werd berekend als het product van de per minuut uitgepompte hoeveelheid vloeistof en de gemiddelde aortadruk (verminderd met P_{lvf}). Om de maximale waarde van W_{lv} (W_{lv}(max)) - onder de gebruikte experimentele omstandigheden - te bepalen, werd P_{lvf} stapsgewijs verhoogd en W_{lv} bij elke waarde van P_{lvf} bepaald. W_{lv} uitgezet tegen P_{lvf} geeft de functiecurve van de linkerkamer. Omdat de maximale waarde van de functiecurve (W_{lv}(max)) door perfusie met adrenaline kan worden verhoogd (waarbij P_{lvf} waarbij W_{lv}(max) wordt bereikt niet wordt beïnvloed), werd W_{lv}(max) als maat voor de contractiliteit van de hartspier gebruikt. Een toename in W_{lv} (max) betekende dus een toename in contractiliteit.

Tijdens de experimenten werden de harten electrisch gestimuleerd met een frekwentie van 360 min^{-1}. De meeste experimenten eindigden binnen 45 min na isolatie van het hart.

Voeding met de genoemde vetten gaf geen onderlinge verschillen in lichaamsmassa, hartmassa en spontane frekwentie van het geïsoleerde hart. De spontane frekwentie was in het begin van de perfusie ongeveer 330 min^{-1}.

$\dot{Q}_{cor}$ in het volgens Langendorff geperfundeerde hart werd door het type voedingsvet beïnvloed. Bijvoorbeeld, na voeding met SSO in vergelijking met L of HCO was $\dot{Q}_{cor}$ tijdens de stationaire perfusie-omstandigheden ongeveer 15% hoger. Deze verhoging werd al na een week van voeding met de vetten gevonden. Na voeding van mengsels van SSO en HCO nam $\dot{Q}_{cor}$ recht evenredig toe met de hoeveelheid SSO in het dieet. In het werkende hart, verhoogde voeding met SSO W_{lv} (max), t.o.v. W_{lv}(max) verkregen na voeding met HCO of L, met ongeveer 10%. Het verschil met de HCO groep werd reeds na een week en met de L groep na vier weken van voeding met de vetten gevonden. Na voeding van mengsels van SSO en L werd er een recht evenredig verband gevonden tussen de verhouding linolzuur/verzadigde vetzuren in de diëten en W_{lv}(max). Daar in zonnebloemolie ongeveer 70% van de vetzuren linolzuur is, werd onderzocht of linolzuur de oorzaak was van de toename in W_{lv}(max). Hiertoe werden twee diëten samengesteld: één met 20 en% TLG + 5 en% TPG en één met 5 en% TLG + 20 en% TPG. Na vier weken van voeding met het eerste dieet was W_{lv}(max) 11% hoger.

Na voeding van diëten met SSO, HCO of L was er in het geïsoleerde hart tussen de groepen geen verschil in zuurstofopname (per eenheid van arbeid), glucose-opname, afgifte van melkzuur en langketen vetzuren. Ook werd geen verschil gevonden in de snelheid van ATP synthese en de ADP/O verhouding in geïsoleerde hartmitochondria. Deze resultaten duiden er op dat de geteste voedingsvetten de substraatverbranding in het geïsoleerde hart niet hadden beïnvloed.

Op grond van de volgende overwegingen werd onderzocht of de gevonden

verschillen in $\dot{Q}_{cor}$ en W_{lv} (max) verklaard konden worden door een effect van de hoeveelheid linolzuur in het dieet op de prostaglandinesynthese in het geïsoleerde hart:

1. De hoeveelheid linolzuur in het voedsel beïnvloedt $\dot{Q}_{cor}$ en W_{lv}(max).
2. Linolzuur is de grondstof voor de prostaglandines van de 1- en 2-serie.
3. Prostaglandines beïnvloeden $\dot{Q}_{cor}$ en de contractiekracht in het geïsoleerde hart.

Allereerst werd nagegaan welke prostaglandines (PG) in het geïsoleerde hart worden gemaakt en hoeveel. Gevonden werd, dat zowel uit het volgens Langendorff geperfundeerde als uit het werkende hart PGD_2 (5%), PGE_2 (15%), $PGF_{2\alpha}$ (5%), PGI_2 (70%) en thromboxaan B_2 (5%) kwamen; de PGI_2 afgifte bedroeg onder stationaire perfusie-omstandigheden ongeveer 8 pmol min^{-1} ($\dot{Q}_{cor}$ ± 15 ml min^{-1}). In beide hartpreparaten was de totale afgifte van prostaglandines gelijk. Alle genoemde prostaglandines verhoogden $\dot{Q}_{cor}$. PGE_2 en PGI_2 deden dit bij zeer lage concentraties (0.4 - 0.6 pmol ml^{-1}). Uit deze gegevens blijkt, dat de hoeveelheid PGI_2 die in het hart wordt geproduceerd ongeveer gelijk is aan de hoeveelheid die nodig is voor vaatverwijding. Bovendien werd door volledige remming van de prostaglandinesynthese $\dot{Q}_{cor}$ verlaagd, waaruit geconcludeerd kan worden dat in het volgens Langendorff geperfundeerde rattehart $\dot{Q}_{cor}$ wordt beïnvloedt door de prostaglandinesynthese. In het werkende hart verhoogde alleen $PGF_{2\alpha}$ (9 pmol ml^{-1}) W_{lv}(max) en had volledige remming van de prostaglandinesynthese geen effect op W_{lv} (max). Vervolgens werden de effecten van diëten met 3 en% en 24 en% linolzuur onderzocht op de prostaglandinesynthese: in het volgens Langendorff geperfundeerde en in het werkende hart onder stationaire perfusie-omstandigheden (basale prostaglandinesynthese) en in het volgens Langendorff geperfundeerde hart onmiddellijk na het begin van de perfusie (gestimuleerde prostaglandinesynthese). Na voeding van beide diëten was de basale prostaglandinesynthese ongeveer gelijk (mogelijk was na voeding met 24 en% linolzuur de basale prostaglandinesynthese enigszins lager). De gestimuleerde prostaglandinesynthese was door het linolzuurrijke dieet enigszins verhoogd (statistisch niet significant).

Mogelijke verklaringen voor de gevonden verschillen in $\dot{Q}_{cor}$ en W_{lv}(max) werden gevonden in de volgende experimenten. Na voeding met SSO, HCO en L was het verloop van $\dot{Q}_{cor}$ met de tijd voor de drie groepen verschillend. Deze verschillen waren echter verdwenen na remming van de prostaglandinesynthese. Aangezien de PGI_2 afgifte in de SSO groepen alleen tijdens het begin van de perfusie (gestimuleerde prostaglandinesynthese) enigszins hoger was dan die van de twee andere groepen, is het mogelijk dat door verschillen in de gestimuleerde prostaglandinesynthese verschillen in het verloop van $\dot{Q}_{cor}$ ontstonden. Voeding met SSO in vergelijking met HCO en L verlaagde de

Na^+,K^+-ATPase activiteit in het hart met ongeveer 10% (statistisch niet significant). Door sterke verlaging gedurende twee weken van het schildklierhormoon in het plasma, verdween het verschil in W_{lv}(max) tussen SSO en L gevoede groepen. Aangezien het schildklierhormoon de Na^+, K^+-ATPase activiteit van de hartspier beïnvloedt is het mogelijk dat het effect van de hoeveelheid linolzuur in het voedsel op de contractiliteit van de hartspier via het schildklierhormoon verliep.

Geconcludeerd kan worden dat: 1) de hoeveelheid linolzuur in het voedsel de regulatie van de coronaire doorstroming in het geperfundeerde rattehart beïnvloedt; dit gebeurt misschien door een effect op de prostaglandinesynthese in het begin van de perfusie en 2) er een positieve, lineaire relatie aanwezig is tussen de linolzuur / verzadigde vetzuren verhouding in het voedsel en de contractiliteit van de hartspier. Deze laatste beïnvloeding komt misschien tot stand door een invloed op de Na^+, K^+-ATPase activiteit in de hartspier. Voor de extrapolatie naar de mens is het van belang de oorzaken van het effect van de hoeveelheid linolzuur in het voedsel op de coronaire stroomsterkte en contractiliteit van de hartspier verder te bestuderen.

ADDENDA

I E.A.M. de Deckere and F. ten Hoor. A modified Langendorff-technique for metabolic investigations. *Pflügers Arch.* **370**, 103-105, 1977.

II E.A.M. de Deckere, D.H. Nugteren and F. ten Hoor. Prostacyclin is the major prostaglandin released from the isolated rabbit and rat heart. *Nature* **268**, 160-163, 1977.

III E.A.M. de Deckere and F. ten Hoor. Effects of dietary fats on coronary flow rate and the left ventricular function of the isolated rat heart. *Nutr. Metab.* **23**, 88-97, 1979.

IV E.A.M. de Deckere and F. ten Hoor. Influences of dietary fats on coronary flow rate and left ventricular work of the isolated rat heart; sunflower seed oil versus lard. *Nutr. Metab.* **24**, 396-408, 1980.

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ADDENDUM I

A Modified Langendorff Technique for Metabolic Investigations*

E. A. M. De DECKERE and P. TEN HOOR

Unilever Research, Postbus 114, Vlaardingen, The Netherlands

SUMMARY

A small amount of fluid keeps dripping from the apex of the isolated, perfused heart, after cannulating the pulmonary artery and carefully preventing leakage from caval and pulmonary veins. This fluid has a high absorbance at 280 nm, which points to a high protein content; it shows a fall in glucose, and a rise in lactate concentration almost corresponding with the heart's total glucose uptake and lactate release respectively. The data suggest that a small amount of the perfusion fluid reaches the surface of the heart via the interstitial space.

Since its introduction by Langendorff[1] the isolated perfused heart has been frequently used as an experimental set-up in pharmacology, physiology and biochemistry. The main advantages of the method are that the heart can be kept beating for hours, that it is easily accessible for a variety of measurements and that chemically defined media can be used for perfusion. When the isolated perfused heart is used for metabolic investigations, changes in concentration of various constituents of the perfusion fluid must be determined. As, in general, these concentration changes are small after a single passage of the perfusate through the heart, recirculation of the perfusion fluid, resulting in larger concentration changes and thus improving accuracy, is often applied. However, accumulation of substances released by the heart may then influence the metabolic investigation.

Using a slightly modified Langendorff set-up in rat and rabbit hearts, we observed that about 3% of the perfusion fluid entering the coronary arteries does not follow the normal route of ejection by the right ventricle, but apparently leaves the vascular bed, sips through the heart tissues and thus reaches the surface of the heart. In this part of the perfusion fluid (amount Q_i; flow rate $\dot{Q}_i$) the concentration of a number of substances is much higher than in the fluid ejected by the right ventricle (amount Q_{rv}, flow rate $\dot{Q}_{rv}$) so that it is more suitable for direct measurements.

To prepare this modified Langendorff set-up (Fig. 1) the caval and pulmonary veins are tied off after the heart has been connected to a perfusion apparatus filled with a continuously gassed (O_2/CO_2 = 95/5) Krebs-Henseleit buffer solution supplemented with glucose (11.1 mmol l^{-1}) and EDTA (0.5 mmol l^{-1}). A cannula is then tied into the pulmonary artery to gather Q_{rv}. A small amount of fluid (Q_i) keeps dripping from the heart and is gathered at the apex.

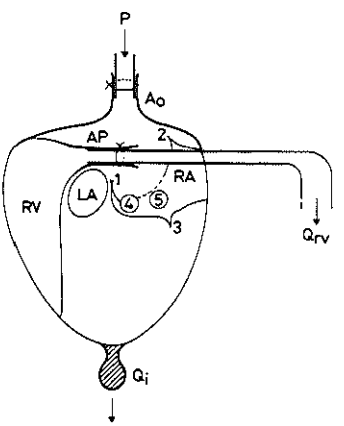

Fig. 1 Schematic representation of modified Langendorff set-up. Aorta (Ao) and pulmonary artery (AP) are cannulated. The left (1) and right (2) superior venae cavae and the inferior vena cava (3) are tied off close to the atrium. The pulmonary veins (4,5) are tied off close to the lungs and the lungs are cut off. The coronary effluent is ejected by the right ventricle (RV) via the cannulated pulmonary artery (Q_{rv}). A small amount of fluid (Q_i) keeps dripping from the apex. The composition of Q_i reflects the metabolic activity of the heart, and is directly accessible for analysis. LA = left atrium; RA = right atrium, P = perfusion fluid.

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Table 1

Absorbance, glucose concentration and uptake, lactate concentration and production, and inosine and hypoxanthine concentration of fluid dripping from the apex (Q_i) and fluid ejected by the right ventricle (Q_{rv}) in the modified Langendorff set-up

	Q_i	Q_{rv}
Absorbance (n=6)		
258 nm	0.25 ± 0.06	0.004 ± 0.0001
280 nm	0.23 ± 0.06	0.005 ± 0.0001
Glucose (n=5)		
concentration (μmol ml^{-1})	10.95 ± 0.10	11.39 ± 0.03
uptake (μmol min^{-1} g^{-1})	1.50 ± 0.40	-
Lactate (n=5)		
concentration (μmol ml^{-1})	0.11 ± 0.02	0
production (μmol min^{-1} g^{-1})	0.37 ± 0.07	-
Inosine (n=4)		
concentration (nmol ml^{-1})	3.0 ± 1.0	3.0 ± 0.9
Hypoxanthine (n=4)		
concentration (nmol ml^{-1})	3.0 ± 0.3	0.7 ± 0.08

Q_i and Q_{rv} were collected in 6 periods of 5 min. The data are those of the first period (15 to 20 min). The data of the other 5 periods do not differ from the listed values. The glucose uptake and the lactate production are calculated from the concentration differences and $\dot{Q}_i$ (3.4 ± 1.1 ml min^{-1} g^{-1}). The glucose concentration of the perfusion buffer was 11.39 μmol ml^{-1}. Inosine and hypoxanthine were determined during hypoxia (Oxygen pressure of perfusion fluid = 150 mmHg).

Experiments were started after initial perfusion for 15 min. The perfusion pressure (P_p) was 80 mmHg and the stimulation frequency 360 min^{-1}. Measured quantities are expressed per g dry weight, the hearts being dried at 80°C for 24 h. The experiments were done in rat hearts. At P_p = 80 mmHg, $\dot{Q}_i$ was 2.6 ± 0.2 ml min^{-1} g^{-1} (n=6); $\dot{Q}_{rv}$ was 78 ± 8 ml min^{-1} g^{-1}.

In a series of experiments, the absorbance at 258 and 280 nm (Zeiss M4-Q III spectrophotometer), the glucose concentration (Beckman Glucose Analyzer), the lactate concentration (Boehringer Biochemical Test Combination), and the concentration of the adenosine derivatives inosine and hypoxanthine (assayed enzymatically[2]) of 5 min samples of Q_i and Q_{rv} were measured for 30 min. The release of inosine and hypoxanthine was studied at an oxygen pressure of the perfusion buffer of 150 mmHg. The results of these experiments are summarized in Table 1.

In 5 hearts Q_{rv} was recirculated for 30 min and the glucose and lactate concentration of the perfusion buffer and of the recirculated Q_{rv} were measured. Recirculation volume was 90 ml at the start and 70 ml at the end of recirculation; the difference of 20 ml was collected as Q_i. In the recirculated fluid the glucose concentration decreased from 11.39 ± 0.03 to 11.36 ± 0.04 μmol ml^{-1}, while the lactate concentration increased from 0 to 5.0 ± 0.3 nmol ml^{-1}, from which a lactate release into Q_{rv} of 0.05 μmol min^{-1} g^{-1} was calculated. The glucose uptake from Q_i was 1.6 ± 0.5 and the lactate release into Q_i was 0.46 ± 0.06 μmol min^{-1} g^{-1}.

The results presented show that in the isolated perfused rat heart after cannulating the pulmonary artery and gathering the fluid ejected by the right ventricle (Q_{rv}), a small amount of fluid (Q_i = about 3% of Q_{rv}) keeps dripping from the apex. The composition of both fluids is quite different (Table 1). The differences in absorbance at 258 and 280 nm[3] indicate that Q_i contains much more protein than Q_{rv}. A most remarkable observation is that the heart takes up glucose mainly from Q_i and that about 90% of its lactate production is released into this fluid. The concentrations of the adenosine derivatives inosine and hypoxanthine are about the same in Q_i and significantly different in Q_{rv} ($P_2 < 0.05$).

However, as Q_i and Q_{rv} were 12 and 92 ml min^{-1} g^{-1} respectively, it can be calculated that the release of inosine + hypoxanthine into Q_i and Q_{rv} was about 80 and 320 nmol min^{-1} g^{-1} respectively. This may indicate that adenosine is preferentially produced in the blood vessels, which corresponds with the observation that 5'-nucleotidase (which forms adenosine from AMP) is predominantly located in the endothelial[4] and perivascular cells[5] of the capillaries.

The described modification of the Langendorff technique may give more insight into metabolic events occurring in the isolated heart. Moreover, as the fluid dripping from the apex is highly concentrated, concentration changes of substances (e.g. enzymes, hormones) released by the heart in small amounts are easily detected[6].

Acknowledgement

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ADDENDUM II

PROSTACYCLIN IS THE MAJOR PROSTAGLANDIN RELEASED FROM THE ISOLATED PERFUSED RABBIT AND RAT HEART*

Prostacyclin (prostaglandins X, PGI_2), a newly discovered prostaglandin[1-3], is formed by microsomes from several organs, such as pig and rabbit aorta[2] and rat stomach[4], and by fresh human arterial and venous tissue incubated with PGH_2 (ref. 5). As inhibitor of blood platelet aggregation, it is 30 times more potent than PGE_1 (ref. 2) and could thus help in preventing the formation of intra-arterial thrombi. It has been suggested[1] that the endoperoxides released by the platelets can be converted into prostacyclin by vascular tissue. Prostacyclin is chemically unstable in aqueous solution: it hydrolyses to 6-oxo-$PGF_{1\alpha}$ (refs 3, 4; Fig. 1) which does not inhibit platelet aggregation[2]. We report that isolated perfused rabbit and rat hearts produce a labile factor which strongly inhibits blood platelet aggregation; the release of this substance is abolished by indomethacin. Using a physicochemical method, we found 6-oxo-$PGF_{1\alpha}$ in substantial amounts in the perfusates. These findings indicate that prostacyclin is the major prostaglandin released from the heart.

Hearts were perfused with a Krebs-Henseleit buffer solution according to Langendorff or as modified by De Deckere and Ten Hoor[12]. The latter method is based on the observation that in the isolated perfused heart a small part (2-5%) of the perfusion fluid reaches the surface of the heart through the interstitial space and the lymphatics. This smaller flow is separated from the main flow by tying off the veins of the right and left atrium and cannulating the pulmonary artery. Most of the perfusion fluid is ejected by the right ventricle through the cannula (Q_{rv}), a small amount passes the interstitium and drips from the heart (Q_i). As the flow rate of Q_i is small, the concentrations of substances released into Q_i are relatively high.

Prostacyclin-like activity was determined direct in Q_i by measuring the inhibition of ADP-induced rat platelet aggregation (Fig. 2)[6]. The biological activity was standardised against PGE_1. As rat platelets are insensitive to PGD_2 and PGE_2, interference from 'stable' prostaglandins in Q_i cannot be expected. PGE_2-like activity was assayed on the isolated gerbil colon[7] in ethyl acetate extracts of acidified perfusates. Antagonists were used as described

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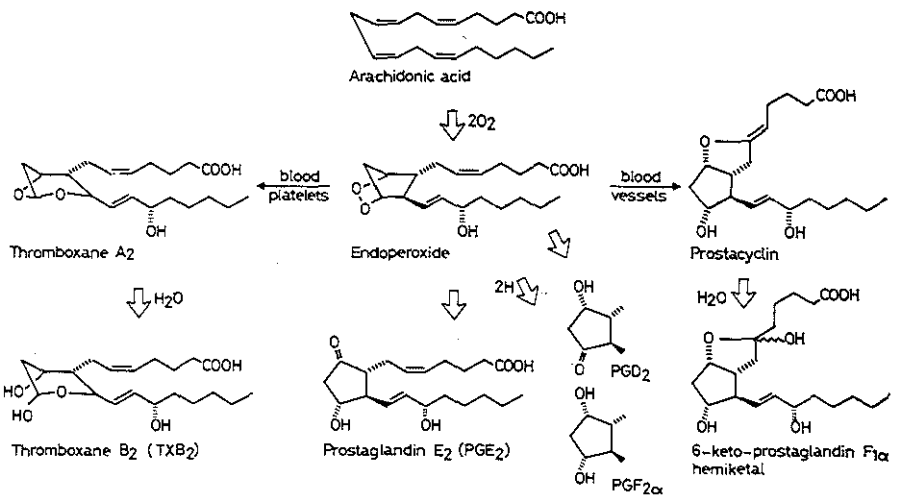

Fig. 1 Routes in the metabolism of prostaglandin-endoperoxide

previously[8]. PGE_2, PGD_2, $PGF_{2\alpha}$, TXB_2 (thromboxane B_2) and 6-oxo-$PGF_{1\alpha}$ (Fig. 1) were extracted, purified by thin-layer chromatography as their methoximated pentafluorbenzyl esters and, after silylation, determined by gas chromatography with electron-capture detection[9] (Table 1). Figure 3 gives examples of the results of the determination of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ The isolated perfused rabbit heart released at least twice as much 6-oxo-$PGF_{2\alpha}$ as PGE_2, the amount (8-12 ng per min per g dry weight) being more than that of all other prostaglandins together (Table 2). The results of the bioassay on the gerbil colon and the gas-chromatographic determinations of PGE_2 correlate reasonably well. Since 6-oxo-$PGF_{1\alpha}$ is the stable hydrolysis product from prostacyclin, it is most probably prostacyclin that was released from the isolated rabbit heart. Freshly collected Q_i showed a distinct inhibition of platelet aggregation (Fig. 2 and Table 3), an ability which disappeared after incubating Q_i for 10-15 min at 37 °C. Perfusion of the heart with indomethacin (1 μg ml^{-1}) completely abolished the anti-aggregatory activity of the perfusate; annoxia for 6 min increased this activity only moderately. Because of the much greater dilution, no inhibition of platelet aggregation could be detected with Q_{IV}, although in this perfusate 6-oxo-$PGF_{1\alpha}$ was present according to gas chromatography.

Table 1 R_f values of the methoximated (MO) pentafluorobenzyl (PFB) esters after TLC and retention times (R_t) of the trimethylsilylated (TMS) derivatives during GLC

Compound*	%†	R_f (MO·PFB)	R_t‡ (MO·PFB·TMS) (min)	Compound*	%†	R_f (MO·PFB)	R_t‡ (MO·PFB·TMS) (min)
PGD_2	90	0.69	16.0	Thromboxane B_2	43	0.46	19.8
	10	0.57	14.7	(TXB_2)	57	0.38	19.8
PGE_2	74	0.54	17.5	6-oxo-$PGF_{1\alpha}$	58	0.37	22.0
	26	0.46	15.2		42	0.31	22.2
ω-nor-PGE_2	68	0.53	14.2	$PGF_{2\alpha}$	—	0.28	15.2
	32	0.45	12.3	$PGF_{1\alpha}$	—	0.25	17.8

100 ng ω-nor-PGE_2 (ref. 10) and 100 ng $PGF_{1\alpha}$ in 1 ml methanol were added to the perfusates as internal standards. (For the experiment of Table 2, the two standards were added after extraction.) The solution was then acidified with HCl (6 mol l^{-1}), drop by drop, until *p*H 4.0. The perfusates were extracted twice with equal volumes of ethyl acetate. The extract was taken to dryness *in vacuo* at 30 °C and the residue rinsed with 2–6 ml methanol into a small test tube. The solvent was removed with nitrogen, and 0.2 ml 2% methoxyamine HCl in pyridine was added. After 16 h at 20 °C, the greater part of the pyridine was removed with nitrogen; 0.5 ml H_2O was added and the prostaglandins were extracted with 2×2 ml ether. To the residue of this extraction, 30 µl pentafluorobenzylbromide was added followed by 60 µl 10% diisopropylethylamine in acetonitrile[9]. After standing for 1 h at 20 °C, the solvents were again removed with a nitrogen stream. The residue was applied as a 2-cm band on a 0.25-mm thick silica gel Fertigplatte (Merck) and developed with chloroform–methanol (90:6, v/v). The appropriate bands were scraped off the plate and eluted with 1–2 ml ether–methanol (2:1, v/v). The eluates were evaporated, the residue was silylated with 25 µl *bis*-(trimethylsilyl)-trifluoroacetamide–pyridine (1:1, v/v) for 1 h at 20 °C and excess reagent removed with nitrogen. Between 0.5 and 5 ng of the PFB esters was injected for gas chromatography, giving sufficiently large peaks with the electron-capture detector.

*Authentic prostaglandins used as references and standards were obtained by biosynthesis from the corresponding unsaturated fatty acids or by incubating PGH_2 with blood platelets (TXB_2) or with sheep aorta microsomes (6-oxo-$PGF_{1\alpha}$).

†The methoximes were present as two isomers (*syn* and *anti*) in the ratio as indicated. $PGF_{2\alpha}$ and $PGF_{1\alpha}$ cannot give a methoxime.

‡2% SE 30, $l = 150$ cm, $d = 0.2$ cm, at 230 °C; carrier gas: argon–10% methane, 25 ml min^{-1}. A Hewlett–Packard 5700 A gas chromatograph with a linear electron capture detector 18713 A was used.

TABLE 2 Prostaglandin release (ng per min per g dry weight) from the isolated, perfused rabbit heart after annoxia

Group	Gerbil colon	Gas chromatography				
	PGE_2-like activity	PGE_2	PGD_2	$PGF_{2\alpha}$	TXB_2	6-oxo-$PGF_{1\alpha}$
1 (n=8)	4.7 ± 0.6	3.5	0.9	1.8	2.2	9.3
2 (n=8)	3.3 ± 0.5	3.9	1.2	1.8	2.1	9.9

In two separate experiments, eight rabbit hearts (about 0.7 g dry weight) were perfused according to Langendorff (for details, see Table 3). Fifteen minutes after starting the perfusion, the hearts were perfused with an oxygen-free buffer for 3 min. The buffer was then reoxygenated and the perfusion fluid collected for 10 min to give about 400 ml perfusate per heart. This fluid, after acidification, was extracted with ethyl acetate (Table 1). Aliquots of the extracts were tested individually for PGE_2-like activity; PGE_2 served as standard. The other prostaglandins found in the perfusate have relatively little activity on the gerbil colon. The remaining parts of the samples were pooled in the two groups of eight and the five prostaglandins determined in triplicate.

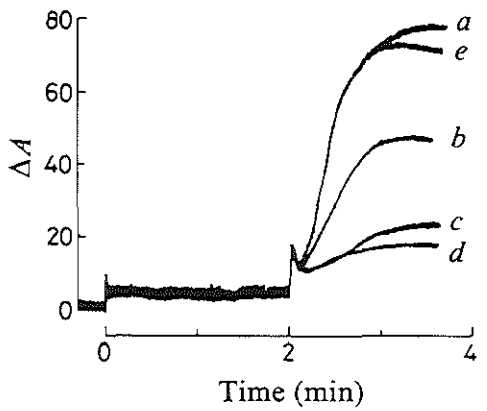

Fig. 2 Inhibition of ADP-induced rat platelet aggregation by freshly collected interstitial effluent Q_i from the isolated perfused heart. Changes in absorbance (Δ A) of rat-platelet-rich plasma (PRP; final volume 3 ml) in a modified Vitatron photometer at 37 °C. At t=0, 0.2 ml buffer solution (Krebs–Henseleit) (*a*), 0.2 ml buffer solution with a PGE_1 concentration of 60 (*b*) or 120 ng ml^{-1} (*c*), 0.2 ml of Q_i (*d*) or 0.2 ml of Q_i heated at 37 °C for 15 min (*e*) was incubated with PRP for 2 min. ADP (final concentration 94 ng ml^{-1}) was then added to induce aggregation. Tracings have been superimposed. As prostacyclin is assumed to be 30 times more potent than PGE_1 (ref. 2), it can be calculated that in this case Q_i contains 4.5 ng ml^{-1} prostacyclin. Tracing *e* shows that heating of Q_i abolishes its anti-aggregatory activity.

Table 3 Prostacyclin and prostaglandin release (ng per min per g dry weight) from the isolated perfused rabbit and rat heart

		Rabbit				Rat		
	Perfusate	Prostacyclin	6-oxo-$PGF_{1\alpha}$	PGE_2	PGD_2	$PGF_{2\alpha}$	TXB_2	6-oxo-$PGF_{1\alpha}$
Before anoxia	Q_{rv}	—	2.7	2.9	< 0.5	0.5	0.6	2.2
	Q_l	4.2	4.0	0.5	< 1	0.4	0.3	3.1
Following anoxia	Q_{rv}	—	2.2	1.2	n.d.*	0.6	n.d.*	1.8
	Q_l	6.2	8.7	0.7	n.d.*	0.2	n.d.*	1.7

Hearts were perfused with a Krebs–Henseleit buffer solution, gassed with O_2–CO_2 (95:5) and supplemented with glucose (11.1 mmol l^{-1}) and EDTA (0.5 mmol l^{-1}), by the modified Langendorff technique of De Deckere and Ten Hoor[12]. Perfusion pressure was 60 mmHg for rabbit hearts, 80 mmHg for rat hearts; stimulation frequencies were 250 and 360 min^{-1}. Twenty minutes after starting the perfusion Q_{rv} and Q_l were collected for 20 min from two rabbit hearts (about 1 g dry weight per heart; hearts were dried at 80 °C for 24 h; dry weight was about 20% of wet weight) and four rat hearts (about 0.2 g dry weight per heart) to give a total of 1400 ml Q_{rv} and 22 ml Q_l from the rabbit hearts and 1,200 ml Q_{rv} and 30 ml Q_l from the rat hearts. Next, the rabbit hearts were perfused with a buffer, gassed with N_2–CO_2 (95:5) for 6 min, the rat hearts for 5 min. Following this anoxic period, the perfusion fluid was reoxygenated and Q_{rv} (rabbit 850 ml, rat 600 ml) and Q_l (rabbit 40 ml, rat 20 ml) were collected for 10 min. Prostacyclin-like activity was determined direct in fresh Q_l samples collected on ice under N_2–CO_2 (95:5), using the blood platelet aggregation assay[6] and assuming an activity of 30 times that of PGE_1 (ref. 2). Prostaglandins were determined by gas chromatography.

*n.d., Not determined.

Perfused rat hearts also released 6-oxo-$PGF_{1\alpha}$ (Table 3) as the major prostaglandin, but anoxia did not increase prostaglandin output. In ten rat hearts Q_i was collected for 5 min (from 20 to 25 min after starting the perfusion). The prostacyclin-like activity was determined by the platelet aggregation test, the prostacyclin production being 3.3 ± 0.4 ng per min per g dry weight. In four of ten hearts, the prostacyclin release, observed for 1 h at 15 min-intervals, was nearly constant. As the release of prostacyclin into Q_i and Q_{rv} is about the same, the total release of prostacyclin from the rat heart can be estimated from the amount released into Q_i.

Our results show that prostacyclin is the major prostaglandin released from perfused rabbit and rat hearts. The negligible amount of thromboxane B_2 found in the perfusion fluid renders it likely that there are hardly any blood platelets present in the isolated perfused heart. This indicates that the heart can synthesise prostacyclin directly from arachidonic acid. We also found that the amount of prostacyclin released from the heart is almost constant during perfusion for more than 1 h, and that it is abolished by infusion of indomethacin (1 μg ml^{-1}). Moncada and co-workers found that in rabbit and pig aortas[1] and in human arteries and veins[5] prostacyclin is almost exclusively synthesised from prostaglandin endoperoxides. They suggest that *in vivo* endoperoxides originating from blood platelets, in active contact with the endothelial layer, are converted into prostacyclin. Our results show that the rabbit and rat heart can synthesise prostacyclin in the absence of blood platelets.

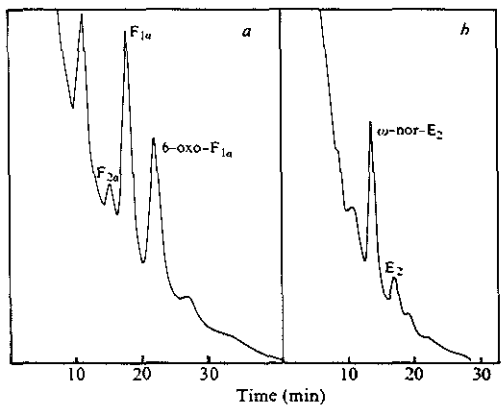

Fig. 3 Gas-chromatographic results of the determination of $PGF_{2\alpha}$ (*a*), 6-oxo-$PGF_{1\alpha}$ (*a*) and PGE_2 (*b*) in rabbit heart perfusates. The methoxime-pentafluorobenzylester-trimethylsilylether derivatives were used for the electron-capture detection. $PGF_{1\alpha}$ (100 ng) and ω-nor-PGE_2 (100 ng) had been added as internal standards. Attenuation × 128. For experimental details, see Table 1.

The physiological consequences of these findings cannot yet be estimated. The continuous production of prostacyclin by the heart and the anti-aggregatory and vasodilatory activity suggest that the main function of prostacyclin production is to protect the coronary circulation against the formation of blood platelet aggregates, thus preventing the occlusion of small branches of the vulnerable coronary tree. If the present results are extrapolated to the human heart, prostacyclin could be a key substance with respect to the origin[11] as well as the prevention of coronary diseases.

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E.A.M. DE DECKERE
D.H. NUGTEREN
F. TEN HOOR

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Unilever Research,
Vlaardingen,
The Netherlands

ADDENDUM III

Effects of Dietary Fats on the Coronary Flow Rate and the Left Ventricular Function of the Isolated Rat Heart*

E.A.M. de Deckere and F. ten Hoor
Unilever Research, Vlaardingen

Key Words. Dietary fat · Isolated rat heart · Coronary flow rate · Left ventricular work · Metabolism

Abstract. Two groups of rats were fed diets containing large amounts (45–50% of the total digestible energy) of sunflower seed oil or hydrogenated coconut oil for 4–5 days. The left ventricular working capacity, the coronary flow rate, the oxygen consumption, the glucose uptake and the lactate release were determined in the isolated perfused heart. The fatty acid composition of the heart phospholipids was also determined. The left ventricular working capacity and the coronary flow rate of hearts of rats fed sunflower seed oil are higher (10–20%) than those of rats fed hydrogenated coconut oil. Feeding the two fats for 3–4 weeks instead of 4–5 days does not alter the results. There are no or only minor differences between the two dietary groups as to the other quantities mentioned.

It is concluded that dietary fats affect the properties of the heart already after a short feeding time.

Introduction

The influences of diets on coronary atherosclerosis have been studied extensively. Diets, however, influence the heart not only by causing atherosclerosis. Erucic acid, for instance, fed to rats for 3 days or 3 weeks, lowers the contractile force of the isolated papillary muscle and the left ventricular stroke work in the heart-lung preparation (24). The objective of the present investigation was to study in which way other than by causing coronary atherosclerosis dietary fats can affect the function of the heart. To this end sunflower seed oil (high in linoleic acid) and hydrogenated coconut oil (high in saturated fatty acids) were fed to rats for a short period. The effect of the dietary fats on the coronary flow rate, the oxygen consumption and the working capacity of the isolated heart were studied. A high amount of dietary fat (45 or 50% of the total digestible energy) was used, as it was thought that this might provoke more distinct effects.

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Materials and Methods

Animals, Diets and Feeding Procedure

Groups of male Wistar rats, which had free access to food and water, were given the diets listed in table I, plus sufficient vitamins and minerals. The fatty acid composition of the two fats in the diets is given in table II. The rats received normal laboratory chow (Muracon) before the diets were fed to them. Details of the feeding procedure and the effects studied are listed in table III.

Isolated Rat Hearts

Rats were anaesthetized (sodium pentobarbital 60 mg/kg, intraperitoneally), heparinized (30 mg/kg, intravenously) and decapitated. Hearts were excised and perfused with Krebs-Henseleit buffer (15) supplemented with 11.1 mmol/l glucose, 0.5 mmol/l EDTA (17) and 17.5 mg/l heparin. The perfusion fluid (38 °C) was gassed with 95% O_2 + 5% CO_2. The oxygen pressure of the perfusion fluid was about 650 mm Hg. The starting perfusion pressure (P_p) was 55 mm Hg. The perfusion apparatus was that described by *Zimmerman* (26), modified for the working rat heart perfusion (17).

Determination of the Left Ventricular Function of the Working Heart

Following 15 min perfusion by the Langendorff method, the hearts were perfused via the left atrium (17); the left ventricle ejected the perfusion fluid against a hydrostatic pressure of 70 mm Hg. The left ventricular external work (J min^{-1} g^{-1}) was calculated from

Table I. Digestible energy (%) of diets

Diet	Sunflower seed oil	Hydrogenated coconut oil	Casein	Starch
A	50	–	23	27
B	45	–	23	32
C	5	45	23	27
D	–	45	23	32

Table II. Main fatty acid composition (wt%) of sunflower seed oil and hydrogenated coconut oil

Fatty acid	Sunflower seed oil	Hydrogenated coconut oil
C 8:0		7
C 10:0		6
C 12:0		45
C 14:0		17
C 16:0	6	10
C 18:0	4	14
C 18:1 (n – 9)	25	
C 18:2 (n – 6)	62[1]	

[1] *Cis-cis*-linoleic acid.

Table III. Details of feeding procedure and dietary effects studied

Effect on	Rats		Dietary group		Feeding period	Results in
	age weeks	body weight g	n	n	days	
Left ventricular function of isolated working heart	25	360	A 8	C 9	4	figure 1
	13	260	A 9	C 9	21	figure 2
Coronary flow rate of Langendorff perfused heart	28	370	A 6	C 6	4	table IV
	13	255	A 8	C 8	4	table IV
	13	250	A 8	C 8	28	Results section
Metabolism in the Langendorff perfused heart	25	360	A 6	C 6	5	table V
Phospholipid fatty acid composition	11	230	B 6	D 6	4	table IV
Influence of dietary aspirin (60 mg/day) on coronary flow rate	17	300	A 6 (control)	A 6 (aspirin)	5	Results section

the mean systolic aortic pressure and the output of the left ventricle as described by *Penpargkul and Scheuer* (18). The peak systolic aortic pressure was estimated from the peak systolic left ventricular pressure. Measurements have shown that the latter is about 2 mm Hg higher than the former. Kinetic work (17) was neglected, as it was less than 1% of the left ventricular external work. The left ventricular external work was stepwise increased by raising the average left ventricular filling pressure every 4 min. Left ventricular function curves were obtained by plotting the left ventricular external work against the left ventricular filling pressure (21). The oxygen consumption (μmol min^{-1} g^{-1}) and the coronary flow rate (ml min^{-1} g^{-1}) were determined as described by *Neely et al.* (17). The pressure in the aorta, the left ventricular pressure and the left ventricular filling pressure (measured in the atrial cannula) were recorded with Statham P23Db pressure transducers. The inner diameters of the aortic and atrial cannulas were 1.2 and 1.5 mm respectively.

Determination of the Coronary Flow Rate of the Langendorff Perfused Heart

15 min after starting perfusion, hearts were perfused for 5 min at 60, 80 and 100 mm Hg successively, the coronary flow rate being measured at each pressure. The hearts of the 13-week-old rats were infused with adenosine (P_p = 100 mm Hg) above the aorta and the coronary flow rate was measured. The final concentration of adenosine was about 5 μmol/l.

Determination of Glucose Uptake, and Lactate and Fatty Acid Release

The glucose uptake, and the lactate and long-chain fatty acid release were studied in the Langendorff perfused heart (P_p = 80 mm Hg) by the perfusion technique described

earlier (6). Glucose was assayed with the Beckman Glucose Analyzer, lactate with a Biochemical Test Combination (Boehringer) and the fatty acids were determined as described by *Laurell and Tibbling* (16).

Determination of the Phospholipid Fatty Acid Composition

15 min after starting perfusion, the hearts were perfused at 80 mm Hg for 15 min and the coronary flow rate was measured. Immediately after perfusion, the apex of the heart was frozen in liquid nitrogen. The lipids were extracted as described by *Bligh and Dyer* (3), the lipid classes being separated by thin-layer chromatography and the fatty acids by gas-liquid chromatography (27).

Additional Data

In all experiments the hearts were stimulated at a frequency of 360/min. The quantities measured are expressed per gram dry heart weight, dry weight being obtained by drying the hearts at 80 °C for 24 h. The Student's t test for paired or unpaired data was used for statistical analyses.

Results

Dietary Fat and Food Consumption, Body Weight and Dry Heart Weight

The food consumption of the various groups of rats was equal, as were the means of the body weights and dry heart weights.

Dietary Fat Influences on Left Ventricular Function, Coronary Flow Rate and Myocardial Oxygen Consumption (Working Heart)

The left ventricular function curves and the relation between the left ventricular work and both the coronary flow rate and the myocardial oxygen consumption, after 4 days of feeding, are shown in figure 1, those after 3 weeks of feeding in figure 2. The results are the same, except that the coronary flow rates in the two experiments differ. For technical reasons, the function curves were not completed. However, the maximum working capacity of the two groups was nearly reached.

Dietary Fat Influences on Coronary Flow Rate (Langendorff Heart)

Already after 4 days of feeding the coronary flow rate is influenced by the type of dietary fat and this influence is stronger in hearts of younger animals than it is in hearts of older animals (table IV). The coronary flows of the two 13-week-old dietary groups respond to adenosine in the same quantitative way. The coronary flow rates of rats fed diets A and C (table I) for 4 weeks were 89 ± 5 and 71 ± 4 ml min^{-1} g^{-1} (P_p = 80 mm Hg; P_2 <0.05).

Metabolic Studies

Feeding diets A and C for 5 days did not lead to differences in oxygen and glucose uptake and in lactate and fatty acid release of the Langendorff perfused

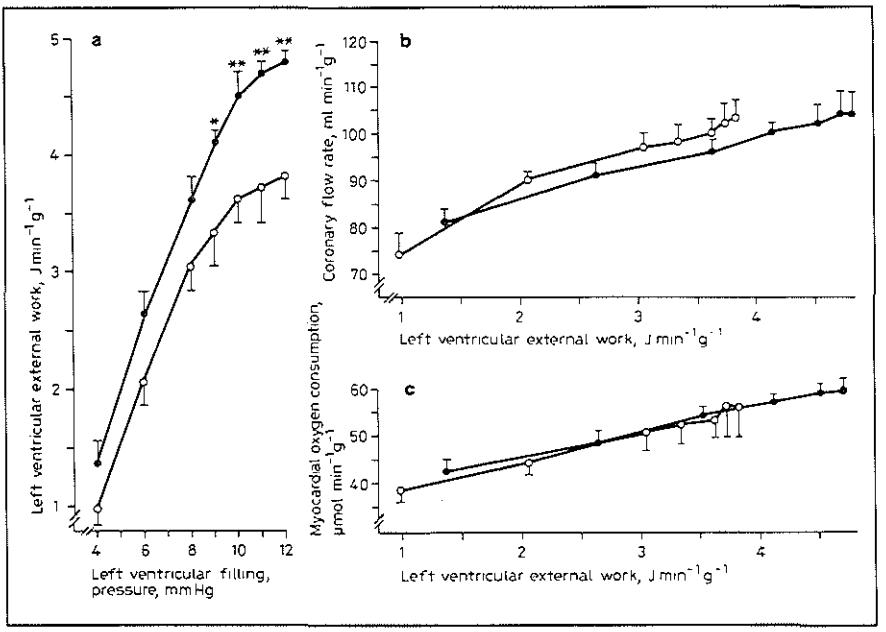

Fig. 1. Influence of diets A (•) and C (○) (see table I), fed to rats for 4 days, on the isolated working rat heart. **a** Relation between left ventricular external work and left ventricular filling pressure. **b** Relation between coronary flow rate and left ventricular external work. **c** Relation between myocardial oxygen consumption and left ventricular external work. Mean values ± SEM; * $P_2 < 0.05$; ** $P_2 < 0.01$.

Table IV. Influence of diets A and C (see table I), fed for 4 days to 13- and 21-week-old rats, on the coronary flow rate (ml min^{-1} g^{-1}) of the Langendorff perfused heart: mean values ± SEM

Perfusion pressure, mm Hg	Age 13 weeks		Age 21 weeks	
	A	C	A	C
60	71 ± 5	56 ± 5	64 ± 4	60 ± 4
		$P_2 < 0.05$	not significant	
80	77 ± 5	62 ± 5	92 ± 10	80 ± 7
		$P_2 < 0.05$	not significant	
100	88 ± 6	71 ± 6	122 ± 14	113 ± 8
		$P_2 < 0.10$	not significant	
100[1]	168 ± 10	178 ± 8		

[1] Adenosine infusion (5 µmol/l).

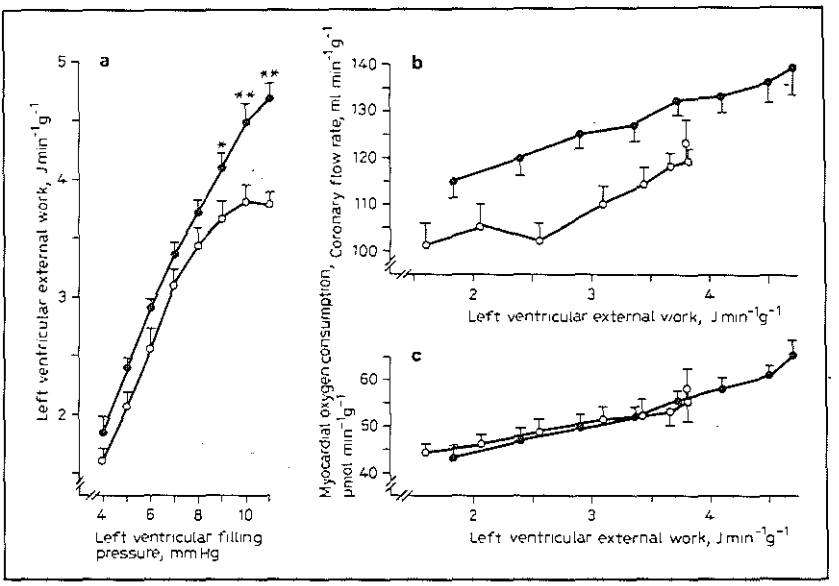

Fig. 2. Influence of diets A (•) and C (○) (see table I), fed to rats for 3 weeks, on the isolated rat heart. For further data, see figure 1.

hearts (table V). The quantities were constant during the perfusion time (three periods of 10 min).

Phospholipid Fatty Acid Composition

The fatty acid composition of the phospholipids of the two groups of hearts of rats fed diets B and D for 4 days was only slightly influenced (table VI). The average coronary flow rate during 15 min perfusion (P_p = 80 mm Hg) of dietary group B was 85 ± 5 ml min^{-1} g^{-1}, that of dietary group D 70 ± 5 ml min^{-1} g^{-1} (P_2 <0.05).

Effect of Dietary Aspirin on Coronary Flow Rate and Heart Frequency

Dietary aspirin had no effect on the coronary flow rate. At a perfusion pressure of 80 mm Hg the coronary flow rate of the control group was 106 ± 4 ml min^{-1} g^{-1}, that of the aspirin group 109 ± 3 ml min^{-1} g^{-1}. Also at perfusion pressures of 60 and 100 mm Hg the coronary flow rates of both groups of hearts were equal. Aspirin lowered the spontaneous frequency of the isolated hearts, that of the control group being 350/min (± 12/min) after 15 min of perfusion, and that of the aspirin group being 287/min (± 12/min). Aspirin did not affect the food consumption of the animals.

Table V. Coronary flow rate, oxygen consumption, glucose uptake, lactate release and long-chain fatty acid release of Langendorff perfused hearts (P_p = 80 mm Hg) of rats fed diets A and C (see table I) for 5 days: mean values ± SEM; the values are the average of three determinations during three periods of 10 min perfusion (15–45 min)

Diet	Coronary flow rate ml min^{-1} g^{-1}	Oxygen consumption μmol min^{-1} g^{-1}	Glucose uptake μmol min^{-1} g^{-1}	Lactate release μmol min^{-1} g^{-1}	Fatty acid release nmol min^{-1} g^{-1}
A	67 ± 3	24 ± 2	2.3 ± 0.3	0.19 ± 0.02	12 ± 2
C	62 ± 2	25 ± 1	2.4 ± 0.4	0.19 ± 0.02	11 ± 2

Table VI. Influence of diets B and D (see table I), fed to rats for 5 days, on the fatty acid composition (%) of the phospholipids of the perfused hearts: SEM values range from 3 to 6%

Fatty acid	Dietary group	
	B	D
C 12:0	0.2	0.4
C 14:0	0.2	0.9
C 16:0	6.7	8.8
C 16:1 (n – 7)	0.7	0.8
C 18:0	19.1	21.1
C 18:1 (n – 9)	5.8	7.2
C 18:2 (n – 6)	25.0	23.5
C 20:4 (n – 6)	15.4	15.6
C 20:5 (n – 3)	1.5	1.0
C 22:4 (n – 6)	1.4	0.9
C 22:5 (n – 6)	0.8	0.4
C 22:5 (n – 3)	2.1	2.1
C 22:6 (n – 3)	10.8	10.4

Discussion

The results show that the coronary flow rate and working capacity of isolated hearts of rats fed sunflower seed oil are higher than those of hearts of rats fed hydrogenated coconut oil; 4 days or 4 weeks of feeding has the same effect. The results concerning the coronary flow rate indicate that in older rats the difference between the two dietary groups is less pronounced than in younger rats, when feeding for 4 days (table IV). This effect was not found for

the working capacity, which indicates that for this criterium an age difference of 12 weeks is probably of little importance. The influence of the age of rats on the results of feeding experiments requires further investigation.

Contractility of the left ventricle was assessed by plotting left ventricular function curves (21), this being a reliable and, in the isolated working heart, applicable method. The function curves of the left ventricle (fig. 1a, 2a) show that at the higher levels of the left ventricular filling pressure the external work of the left ventricle in the sunflower seed oil groups is higher than that in the hydrogenated coconut oil groups. This is caused both by a higher stroke volume and a higher peak systolic left ventricular pressure. The higher stroke volume may be due to a higher compliance or a higher contractility of the hearts of the rats fed sunflower seed oil.

Manifestation of the effects of the two fats already after 4 days of feeding probably indicates that effects are due to metabolic and not to structural changes in the heart. Other authors (12, 25) showed that dietary influences on e.g. the enzyme composition of the liver also reach a maximum as early as after 3–4 days of feeding. As feeding the two fats for 4 days leads to only minor differences in the fatty acid composition of the phospholipids (table VI), changes in the compliance of the heart are not likely.

The finding that the coronary flow rates of the two groups are equal after vasodilatation by adenosine (table IV) indicates that in the Langendorff perfused heart the regulation mechanism of the coronary flow rate is influenced by the dietary fats. The factors which determine the coronary flow rate of the Langendorff perfused heart are not well known. In the working heart, however, the work of the left ventricle is the principal determinant. This study and further investigations (to be published) have shown that in general the differences between the coronary flow rates of the dietary groups are smaller in the working heart than in the Langendorff heart. In one experiment (fig. 2b) the difference was as great as in the Langendorff heart and in another experiment (fig. 1b) the coronary flow rate of the hearts of the rats in the sunflower seed oil group was somewhat smaller than that of the hydrogenated coconut oil group. In the working heart this must be regarded as a normal spread in the results.

Possible explanations for the overall differences between the dietary groups might be found in changes in prostaglandin synthesis or changes in the activity of the Na^+, K^+-ATPase. Dietary fats influence the fatty acid composition of the phospholipids (2, 5, 7–9, 20), which can affect the activity of Na^+, K^+-ATPase (4) or, since the phospholipid fatty acids are the substrates for the prostaglandins (14), the prostaglandin production. However, feeding the two diets for 4 days led only to minor differences in the fatty acid compositions of the phospholipids of the two groups of hearts (table VI). Moreover, insofar as prostaglandins are concerned, dietary aspirin, an inhibitor of prostaglandin synthesis (10, 23), did not affect the coronary flow rate of the isolated hearts.

Although dietary fats can influence the energy metabolism of the heart, the oxygen consumption of the two groups of hearts at a certain level of left ventricular work is equal (fig. 1, 2). As also the glucose uptake and lactate release of the Langendorff perfused hearts are equal (table V), these results do not support an explanation of the found effects via the energy metabolism of the heart.

The working capacity of the isolated hearts can also be influenced by exercise (18, 22) or hormones (11, 13, 19) and may be due to a change in the myosine ATPase activity (1). It is possible that dietary fat or other compounds in the diet influence this ATPase activity directly or hormonally.

Summarizing, dietary sunflower seed oil, compared with hydrogenated coconut oil, increases the coronary flow rate and the working capacity of the isolated rat heart. The mechanism of influencing is still obscure.

Acknowledgement

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ADDENDUM IV

Influences of Dietary Fats on Coronary Flow Rate and Left Ventricular Work of the Isolated Rat Heart: Sunflower Seed Oil versus Lard*

E.A.M. De Deckere and F. Ten Hoor

Unilever Research Laboratory Vlaardingen, Vlaardingen, The Netherlands

Key Words. Dietary fats · Isolated rat heart · Coronary flow rate · Left ventricular work · Isolated mitochondria · Myosin ATPase

Abstract. The influences of dietary sunflower seed oil and lard on coronary flow rate and external left ventricular work were studied in the isolated Langendorff-perfused and working rat heart. For 1, 4 or 6 weeks, rats were fed diets containing 25–50% of the total digestible energy as fat, 23% as casein and 52–27% as starch. The coronary flow rate and the maximum left ventricular work of hearts of rats fed sunflower seed oil were higher than those of hearts of rats fed lard (about 15 and 10%, respectively). The maximum left ventricular work was achieved at a left ventricular filling pressure of 10–12 mm Hg: this value was not affected by the type of dietary fat. The effect of dietary fat on coronary flow rate is already seen after 1 week of feeding, and on left ventricular work after 4 weeks of feeding. Analysis of variance shows a positive relationship between the maximum left ventricular work and the amount of sunflower seed oil. It is concluded that dietary fats affect coronary flow rate and left ventricular work in the isolated rat heart. The increase in left ventricular work may be caused by an increase in contractility.

Introduction

Dietary fats can influence the function of the heart in a number of ways. For example, saturated fats can cause atherosclerosis of the coronary arteries (14), dietary erucic acid promotes accumulation of cardiac lipids followed by development of necrotic foci (1), hearts of rats fed high-erucic acid rapeseed oil for 3 days show a lower performance than hearts of rats fed sunflower seed oil (15), and the coronary flow rate of isolated hearts of rats fed sunflower seed oil

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is higher than that of hearts of rats fed hardened coconut oil (6,8). Moreover, the fatty acid composition of cardiac phospholipids depends on the dietary fat composition (10, 24). Also the ATPase from heart microsomes (12) and the $(Na^+ + K^+)$-ATPase (11) are affected by dietary fats.

To enlarge the knowledge of the effects of dietary fats on the heart, the influence of dietary sunflower seed oil and lard on coronary flow rate, left ventricular work and spontaneous frequency of the isolated rat heart was studied. As vitamin E is an important constituent of sunflower seed oil and as it may affect the heart (13), its effect on the isolated heart was included in the study. The effect of dietary fat on myosin ATPase activity was also determined as it was found that under certain conditions an increase in left ventricular work of the isolated rat heart could be ascribed to an increase in myosin ATPase activity (4). Finally, the respiratory properties of the isolated heart mitochondria were studied, because the rate of ATP synthesis can influence myocardial performance.

Materials and Methods

Animals, Diets and Feeding Procedure

Groups of male Wistar rats were fed diets containing 25–50% of the total digestible energy (en%) as sunflower seed oil or lard, 23% as casein and 52–27% as starch. All diets were supplemented with minerals and vitamins in sufficient amounts (25). Before the experimental period, all rats received Muracon laboratory chow. The animals had free access to food and water. Mean body weight of the groups of rats at the end of the experimental period was between 225 and 250 g, in two experiments 350 g. Details of the experiments and of the effects investigated are given in table I. The fatty acid composition of the fats is given in table II.

Heart Preparation

Rats were anesthetized (sodium pentobarbital 60 mg kg^{-1} i.p.), heparinized (30 mg kg^{-1}, i.v.) and decapitated. Hearts were excised and perfused with a Krebs-Henseleit buffer solution (17) supplemented with 11.1 mmol l^{-1} glucose and 0.5 mmol l^{-1} EDTA (20). The perfusion fluid was filtered with a 3 μm Millipore® filter, and gassed with 95% O_2 + 5% CO_2. The oxygen pressure of the perfusion fluid was about 650 mm Hg. Starting perfusion pressure was 55 mm Hg.

Langendorff-Perfused Hearts

Perfusion apparatus was that by *Zimmerman* (26), the perfusion pressure being increased to 80 mm Hg. 15 min after starting the perfusion, the coronary flow rate ($\dot{Q}_{cor}$) was measured every minute for a period of 10 min by collecting the fluid dripping from the

Table I. Details of feeding procedure and effects studied

Experiment	Group	Digestible energy (en%) supplied by		Feeding time weeks	Effects studied
		SSO	L		
1	1 (6)	50	–	1	coronary flow rate and
	2	–	50		spontaneous frequency in the
2	1 (7)	35	–	6	Langendorff-perfused rat heart
	2	2	33		
3	1 (10)	25	–	1	spontaneous frequency, coronary
	2	35	–		flow rate, myocardial oxygen
	3	45	–		consumption and left ventricular
	4	–	25		work in the isolated working
	5	–	45		rat heart
4	1 (10)	35	–	4	
	2	24	11		
	3	13	22		
	4	2	33		
	5[1]	2	33		
5	1 (10)	30	–	6	
	2	45	–		
	3	2	28		
	4	2	43		
6	1 (9)	35	–	6	myosin ATPase activity
	2	2	33		
7	1 (8)	35	–	6	respiratory properties of the
	2	2	33		isolated heart mitochondria

SSO = Sunflower seed oil; L = lard. Numbers in parentheses are the number of hearts.
[1] Diet supplemented with as much vitamin E as present in the diet containing 35 en% SSO (595 mg vitamin E/kg SSO). In the indication of the groups, the en% of the fat in the diet is given before the abbreviations of the fat. For the composition of the diet see text.

Table II. Fatty acid composition (weight %) of sunflower seed oil (SSO) and lard (L)

Fatty acid	SSO	L
16:0	6.3	25.3
16:1	–	3.2
18:0	4.0	13.4
18:1	18.2	44.5
18:2[1]	70.0	7.9
18:3	0.2	0.7
20:0	0.1	0.4
20:1	0.2	1.0

[1] *(Z)-(Z)*-linoleic acid.

heart in a graduated tube. The average $\dot{Q}_{cor}$ during the 10-min period was used to calculate the mean $\bar{\dot{Q}}_{cor}$ of a group. $\dot{Q}_{cor}$ is expressed in milliliters per minute per gram of dry heart mass.

Working Hearts

For the working heart (20) the perfusion apparatus was modified in that the aorta could be connected via a three-way stopcock to the oxygenating vessel (200 ml) or to an overflow device. The hydrostatic aortic pressure exerted by the water column of the overflow device was 70 mm Hg. An air-filled expansion vessel was placed 7 cm above the aortic valves. The left atrium was connected to the oxygenating vessel via a stopcock and a thermostatted tube. Another overflow device, connected to the thermostatted tube, regulated the mean left atrial pressure or left ventricular filling pressure (P_{lvf}). The perfusion fluid from the aortic and atrial overflow devices was reoxygenated, heated and pumped back into the perfusion reservoir. 15 min after starting the perfusion, hearts were perfused via the left atrium and the aorta was connected to the aortic overflow device. P_{lvf} was increased every 4 min from 6 to 15 mm Hg or from 8 to 13 mm Hg to increase external left ventricular work (W_{lv}). P_{lvf} was measured at the entrance of the left atrium with a Statham P23V pressure transducer. The aortic pressure was measured just above the aortic valves with a Statham P23Db pressure transducer. Portex nylon tubing (inner diameter 0.75 mm) was used for pressure measurements. W_{lv} was calculated from the mean aortic pressure and the output of the left ventricle as described by *Penpargkul and Scheuer* (23). W_{lv} is expressed in Joules per minute per gram of dry heart mass. Kinetic work (20) was neglected, as it was less than 1% of W_{lv}. The inner diameters of the aortic and atrial cannulas were 2.1 and 1.6 mm, respectively. The myocardial oxygen consumption (μmol min^{-1} g^{-1} dry heart mass) was determined as described by *Neely* et al. (20). The spontaneous frequency (min^{-1}) of the heart was measured 10 min after starting perfusion by counting the pulsations in the aortic pressure. During the experiments the hearts were stimulated at a frequency of 360 min^{-1} (10 V, 3 ms). Dry heart mass was obtained by drying the hearts at 80 °C for 24 h.

Myosin ATPase

For two dietary groups the myosin ATPase activity of three pools of three hearts was determined as described by *Bhan and Scheuer* (4). Instead of the inorganic phosphate formed, the ADP formed was measured with a Biochemica Test Combination (Boehringer, Mannheim).

Isolated Heart Mitochondria

Heart mitochondria were isolated according to *Hülsmann* (16). Oxygen consumption (Q_{O_2}) was measured with a Gilson Oxygraph model KM, provided with a micro Clark electrode. Oxygen consumption was determined with and without ADP. From these values, the Respiratory Control Index (RCI), ADP/O and the rate of ATP synthesis were calculated, using glutamate and malate as substrate. Protein was analyzed by the biuret method. The total fatty acid composition of the mitochondria was determined by GLC (27) after extraction (5) and separation of the lipid classes by TLC.

Statistical Analysis

Statistical analysis was performed by Student's t test and analysis of variance.

Results

Effect of Dietary Fats on Body Mass and Dry Heart Mass

In none of the experiments did the various diets lead to differences in the mean body and dry heart masses of the groups. As an example the data obtained in experiment 4 are shown in table III.

Table III. Mean (± SEM; n = 10) dry heart and body mass of rats fed various diets for 4 weeks (exp. 4)

Dietary group	Dry heart mass/mg	Body[1] mass/g
35 SSO	217 ± 3	332 ± 7
24 SSO + 11 L	219 ± 3	342 ± 8
13 SSO + 22 L	219 ± 6	342 ± 7
2 SSO + 33 L	220 ± 4	336 ± 11
2 SSO + 33 L + vitamin E	220 ± 6	336 ± 12

SSO = Sunflower seed oil; L = lard.

[1] The mean body mass at the start of the experimental diets was for each group about 262 g.

Effects of Dietary Fats in the Langendorff-Perfused Heart (Experiments 1, 2)

After 1 week of feeding the 50 sunflower seed oil and 50 lard diets, the $\dot{Q}_{cor}$ values were 99 ± 2 and 87 ± 5 ml min^{-1} g^{-1}, respectively ($p < 0.05$). After 6 weeks of feeding the 35 sunflower seed oil and 33 lard + 2 sunflower seed oil diets the $\dot{Q}_{cor}$ values were 88 ± 5 and 74 ± 4 ml min^{-1} g^{-1}, respectively ($p < 0.05$). The spontaneous frequencies of the latter two groups were 345 ± 13 and 328 ± 11 min^{-1}, respectively ($p > 0.1$).

Effects of Dietary Fats in the Working Heart (Experiments 3–5)

The spontaneous frequencies (table IV) of the hearts of the rats of the sunflower seed oil groups were somewhat higher than those of the lard groups, except when vitamin E was supplemented to the diet.

At W_{lv} between 5.0 and 6.4 J min^{-1} g^{-1}, $\dot{Q}_{cor}$ increased linearly from 104 ± 4 to 121 ± 5 ml min^{-1} g^{-1}; the myocardial Q_{O_2} increased linearly from 57 ± 2 to 71 ± 2 μmol min^{-1} g^{-1}. No differences were found in the values of both $\dot{Q}_{cor}$ and myocardial Q_{O_2} of the dietary groups at the maximal value of W_{lv} (table V).

The effect of dietary sunflower seed oil and lard on the relationship between P_{lvf} and W_{lv} is shown in figure 1A–C (experiments 3–5). Differences in the values of W_{lv} of the dietary fat groups already appear after 1 week of feeding

Table IV. Spontaneous frequencies (f) in the isolated working rat heart (P_{lvf} = 6 mm Hg)

Experiment	Dietary group[1]	f/min^{-1}
4	35 SSO	324 ± 10
	24 SSO + 11 L	320 ± 11
	13 SSO + 22 L	308 ± 15
	2 SSO + 33 L	305 ± 13
	2 SSO + 33 L + vitamin E	324 ± 13
5	30 SSO	321 ± 8
	45 SSO	315 ± 7
	28 L + 2 SSO	303 ± 10
	43 L + 2 SSO	300 ± 7

SSO = Sunflower seed oil; L = lard. Mean values ± SEM, n = 10. In both experiments: $p > 0.05$.

Table V. Mean values (± SEM; n = 10) of the maximum of external left ventricular work (W_{lv}), coronary flow rate ($\dot{Q}_{cor}$) and myocardial oxygen consumption (Q_{O_2}) in the isolated working heart of rats fed various diets

Experiment	P_{lvf}[1] mm Hg	Dietary group	W_{lv} J min^{-1} g^{-1}	$\dot{Q}_{cor}$ ml min^{-1} g^{-1}	Q_{O_2} µmol min^{-1} g^{-1}
3	12	25 SSO	6.06 ± 0.19	117 ± 5	69 ± 2
		35 SSO	6.18 ± 0.15	115 ± 3	70 ± 2
		45 SSO	5.97 ± 0.27	115 ± 4	68 ± 3
		25 L	5.79 ± 0.25	109 ± 3	67 ± 2
		45 L	5.70 ± 0.27	113 ± 4	67 ± 2
4	10	35 SSO	6.38 ± 0.16	118 ± 3	–
		24 SSO + 11 L	6.39 ± 0.18	115 ± 2	–
		13 SSO + 22 L	5.77 ± 0.26	110 ± 3	–
		2 SSO + 33 L	5.91 ± 0.20	109 ± 3	–
		2 SSO + 33 L + vitamin E	5.91 ± 0.19	113 ± 3	–
5	10	30 SSO	6.31 ± 0.16	115 ± 3	65 ± 2
		45 SSO	6.15 ± 0.16	120 ± 3	65 ± 1
		28 L + 2 SSO	5.64 ± 0.07	113 ± 2	63 ± 1
		43 L + 2 SSO	5.69 ± 0.16	114 ± 2	62 ± 1

SSO = Sunflower seed oil; L = lard.

[1] Left ventricular filling pressure at which the maximal value of W_{lv} was obtained.

For statistical evaluation of W_{lv} see figure 1.

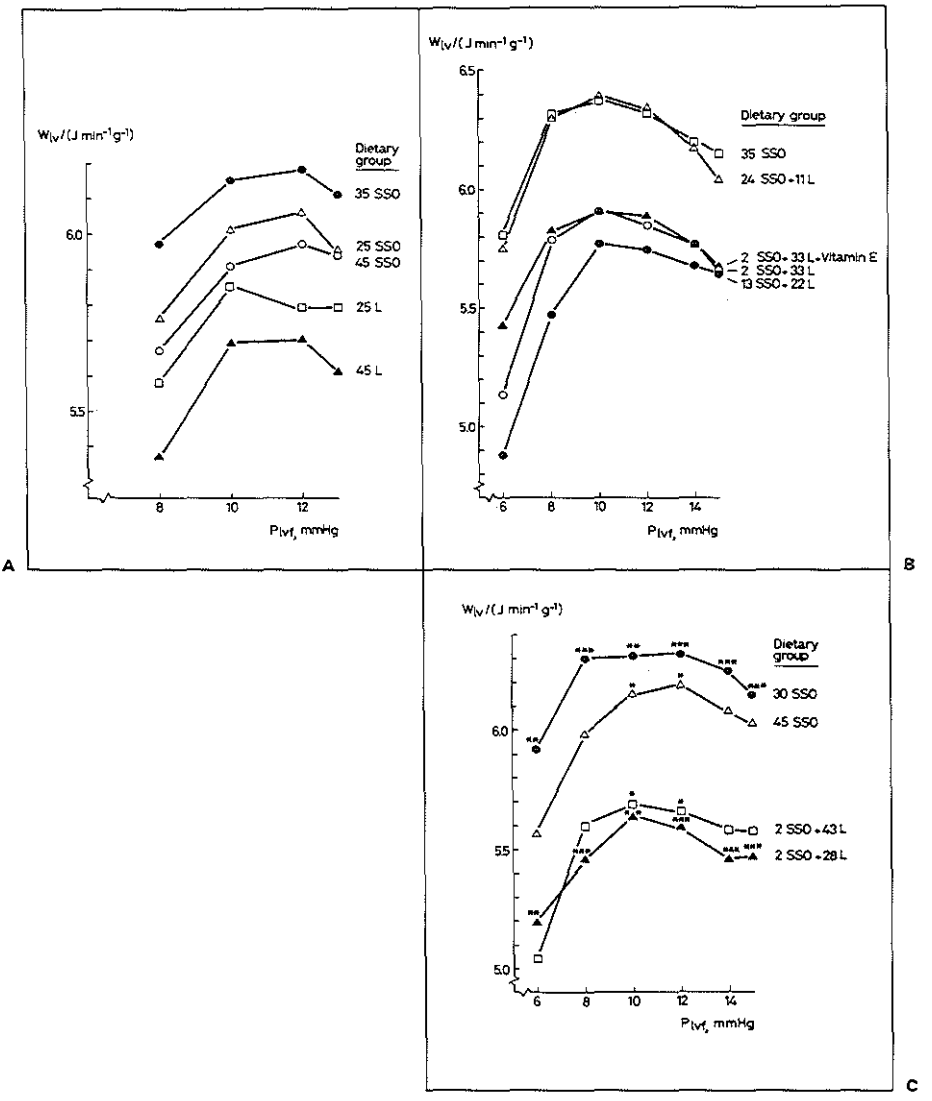

Fig. 1. Influence of dietary sunflower seed oil (SSO) and lard (L) on the relationship between left ventricular filling pressure (P_{lvf}) and external left ventricular work (W_{lv}) of isolated rat hearts. Hearts were stimulated (360 min^{-1}). **A** After 1 week of feeding (experiment 3). W_{lv} values are not significantly different. **B** After 4 weeks of feeding (experiment 4). Analysis of variance (P_{lvf} = 10 mm Hg): W_{lv} = 0.018 (en% SSO) + 5.77 J min^{-1} g^{-1}, $p < 0.05$. **C** After 6 weeks of feeding (experiment 5). 30 SSO vs. 2 SSO + 28 L; 45 SSO vs. 2 SSO + 43 L: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Mean W_{lv} values (n = 10). SEM values ranged from 0.15 to 0.29 (A), 0.13 to 0.26 (B) and 0.07 to 0.22 (C) J min^{-1} g^{-1}. For the dietary groups see table I.

(fig. 1A). After 4 weeks of feeding, the differences are statistically significant (fig. 1B) and are still somewhat greater after 6 weeks of feeding (fig. 1C). The values of W_{1v} of the groups which received 24, 25, 30, 35 and 45 en% sunflower seed oil are similar. The same is true for the groups which received 0, 2 and 13 en% sunflower seed oil. The maximal value of W_{1v} of the latter groups is lower than that of the former groups. In experiment 4 (fig. 1B) analysis of

Table VI. Respiratory properties of isolated heart mitochondria of rats fed 35 en% sunflower seed oil (SSO) or 33 en% lard (L) + 2 en% SSO for 6 weeks (experiment 7) (mean values ± SEM; n = 8)

Parameter	35 SSO	33 L + 2 SSO
Q_{O_2}	325 ± 8	339 ± 12
Respiratory control index	6.70 ± 0.23	6.56 ± 0.14
ADP/O	2.66 ± 0.03	2.67 ± 0.01
ATP synthesis[1]	1,723 ± 32	1,815 ± 67

For the dietary groups see table I. For the 4 parameters: $p > 0.1$.
[1] nmol min^{-1} g^{-1} protein.

Table VII. Fatty acid composition (%) of isolated heart mitochondria of rats fed 35 en% sunflower seed oil (SSO) or 33 en% lard (L) + 2 en% SSO for 6 weeks (experiment 7) (mean values ± SEM; n = 8)

Fatty acid	35 SSO	33 L + 2 SSO
14:0	0.1 ± 0.02	0.3 ± 0.02
16:0	6.7 ± 0.11	9.9 ± 0.11
16:1 (n−7)	0.9 ± 0.04	1.3 ± 0.08
18:0	19.3 ± 0.28	18.0 ± 0.28
18:1 (n−9)	4.6 ± 0.18	7.8 ± 0.19
18:2 (n−6)	30.2 ± 0.65	17.6 ± 0.57
20:3 (n−9)	0.5 ± 0.02	0.4 ± 0.02
20:4 (n−6)	23.0 ± 0.28	22.6 ± 0.51
20:5 (n−3)	0.5 ± 0.04	0.5 ± 0.05
22:4 (n−6)	2.2 ± 0.12	0.5 ± 0.06
22:5 (n−6)	5.1 ± 0.39	1.3 ± 0.10
22:5 (n−3)	0.6 ± 0.02	2.6 ± 0.11
22:6 (n−3)	3.9 ± 0.13	14.1 ± 0.41

variance shows that the maximal value of W_{lv} is related to the amount of dietary sunflower seed oil.

Vitamin E, which is present in high amounts in sunflower seed oil, did not influence W_{lv} (fig. 1B).

Effect of Dietary Fat on Myosin ATPase (Experiment 6) and on Isolated Mitochondria (Experiment 7)

The myosin ATPase activities in the 35 sunflower seed oil and in the 2 sunflower seed oil + 33 lard group were 1.62 ± 0.11 and 1.63 ± 0.04 μmol ADP min^{-1} mg^{-1} protein, respectively. The respiratory properties of the isolated heart mitochondria of these groups were similar (table VI). The fatty acid compositions of the two groups of mitochondria are listed in table VII.

Discussion

The results show that dietary fat can influence spontaneous frequency, $\dot{Q}_{cor}$ and external W_{lv}. The influence of dietary fats on $\dot{Q}_{cor}$ could only be observed in Langendorff-perfused hearts. Feeding sunflower seed oil for 1 week, caused a 15% higher $\dot{Q}_{cor}$ than feeding lard. In the working heart, $\dot{Q}_{cor}$ depended primarily on cardiac work, leading may be to almost maximal coronary vasodilation, so that differences could not be detected (table V). The increase in $\dot{Q}_{cor}$ by feeding diets rich in linoleic acid might be caused by an increase in prostaglandin synthesis. Sunflower seed oil contains about 70% linoleic acid which is the ultimate precursor of the most important prostaglandins. Prostaglandins are potent vasodilators and are released continuously from the isolated rat heart (7). Possibly, feeding diets rich in linoleic acid increased the prostaglandin synthesis in the isolated rat heart as a result of which $\dot{Q}_{cor}$ might have increased. However, this has not been found in similar experiments (9). Moreover prostaglandins are formed from the arachidonic acid in the membrane phospholipids. Table VII shows that after feeding sunflower seed oil and lard the amounts of arachidonic acid in the mitochondria were similar, indicating that the dietary fats did not affect the amount of arachidonic acid in the membranes of the heart. It is also possible that the sensitivity of the coronary vessels for prostaglandins was changed or that other changes in the mechanisms affecting $\dot{Q}_{cor}$ were induced by an increase in prostaglandin synthesis during life time by the diets rich in linoleic acid (22). This has to be investigated.

The increase in external W_{lv} by feeding diets rich in linoleic acid was obtained by both an increase in stroke volume and systolic aortic pressure. The higher stroke volume might be due to a change in the compliance of the left ventricle. However, it can then be expected that the maxima of the external W_{lv} of the sunflower seed oil and lard group were obtained at different values of P_{lvf}. As this was not so (fig. 1), this indicates that diets rich in linoleic acid increased the contractility. This applies when a change in contractility is defined as a change in contractile force or work at equal muscle length (21).

A number of possibilities which can affect myocardial contractile force were investigated for their involvement in the differences in external W_{lv}. $\dot{Q}_{cor}$ (2) and myocardial Q_{O_2} (3) can affect contractile force. However, at the maximal values of W_{lv} both $\dot{Q}_{cor}$ and myocardial Q_{O_2} of the dietary groups did not show statistical differences (table V). The small differences in the values are caused by differences in W_{lv}. As effects of dietary vitamin E on the heart have been reported (13) and as sunflower seed oil is rich in vitamin E the increase in cardiac work after feeding diets rich in sunflower seed oil might be caused by vitamin E. However, adding vitamin E to a diet rich in lard did not affect W_{lv}. The diet-induced increase in W_{lv} is similar to that obtained by training rats (23). In the latter experiments the increase in W_{lv} was found to be due to an increase in myosin ATPase activity (4). Therefore the effect of dietary sunflower seed oil and lard on myosin ATPase activity was investigated. However, differences were not found which rule out this possibility. Neither did the dietary fats affect the respiratory properties of the isolated heart mitochondria by which, possibly, the effect on cardiac work could be explained.

The effect of dietary fats on cardiac work may be explained by the effect of dietary fat on the Na^+,K^+-ATPase activity (11). Myocardial Na^+,K^+-ATPase influences the intracellular Na^+ concentration and consequently the intracellular Ca^{2+} concentration (19) as a result of which the contractile force of the heart is affected (18). This possibility has to be further investigated.

It is concluded that dietary fats influence, $\dot{Q}_{cor}$ and W_{lv} in the isolated rat heart. Assuming that the values of the measured quantities reflect those in the intact heart, dietary fats may influence the performance of the heart *in vivo*.

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E.T.J. Eikema, Editorial Department, Unilever Research Vlaardingen, P.O. Box 114, 3130 AC Vlaardingen (The Netherlands)

ADDENDUM V

INFLUENCE OF TYPE OF DIETARY FAT ON THE PROSTAGLANDIN RELEASE FROM ISOLATED RABBIT AND RAT HEARTS AND FROM RAT AORTAS*

E.A.M. de Deckere, D.H. Nugteren and F. ten Hoor
Unilever Research Vlaardingen, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands

ABSTRACT

It has previously been found (1) that feeding rats a diet containing a high amount of sunflowerseed oil results in a higher coronary flow and left ventricular work in their isolated hearts as compared to hearts of rats fed hydrogenated coconut oil or lard. It was hypothesized that this phenomenon can be explained by an influence of dietary linoleic acid on prostaglandin synthesis in the heart. To verify this hypothesis rabbits and rats were fed for four weeks sunflowerseed oil (SSO), hydrogenated coconut oil (HCO) or lard (L) to a maximum of 30 to 40 per cent of the total digestable energy, and the prostaglandin release from the isolated perfused hearts and rat aortas was determined by gas chromatography and bio-assay (PGI_2).

For the isolated hearts of rabbits fed SSO, the release of PGE_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ was 1.7, 0.7 and 3.0 ng $min^{-1}g^{-1}$ dry weight respectively; when fed L, these values were 2.9, 1.1 and 5.6 ng $min^{-1}g^{-1}$. For the isolated hearts of rats fed SSO, HCO or L, the total release of PGE_2, PGD_2, $PGF_{2\alpha}$ and thromboxane B_2 (TXB_2) was 5.9, 5.8 and 5.6 ng $min^{-1}g^{-1}$ respectively; the release of 6-oxo-$PGF_{1\alpha}$ was 3.4, 5.7 and 6.4 ng $min^{-1}g^{-1}$ respectively. Relatively, 26% PGE_2, 13% PGD_2, 8% $PGF_{2\alpha}$, 6% TXB_2 and 47% 6-oxo-$PGF_{1\alpha}$ were released. For the isolated aortas of rats fed SSO or HCO, the release of PGI_2-like activity was 0.37 ± 0.05 and 0.49 ± 0.05 ng $min^{-1}cm^{-2}$. The release of PGI_2-like activity from hearts of EFA-deficient rats was about 20% of that from control hearts.

We conclude that, although feeding sunflowerseed oil, with respect to feeding hydrogenated coconut oil or lard, does increase coronary flow and left ventricular work, it does not increase the basal prostaglandin production in the isolated rat or rabbit heart; instead there is a tendency for a lower PGI_2 synthesis.

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INTRODUCTION

The coronary flow rate and the left ventricular work of the isolated hearts of rats fed sunflowerseed oil (SSO; containing 65% (w/w) linoleic acid) for three or four weeks are higher than those of the hearts of rats fed hydrogenated coconut oil (HCO) or lard (L) (1). These findings might be explained by the role of dietary linoleic acid, as it is the ultimate precursor of prostaglandins. It is known that PGE_2 and PGI_2 are vasodilatory (2,3) and that $PGF_{2\alpha}$ may increase the contractile force of the heart (4). This explanation implies the assumption that a higher amount of dietary linoleic acid increases the prostaglandin production in the heart (5,6).

In the present study, the influence of dietary fat on the prostaglandin production was investigated. Rabbits and rats were fed SSO, HCO or L, and after four weeks, the hearts and aortas were excised and perfused. The release of prostaglandins into the perfusates was measured by gas chromatography with electron-capture detection or by bio-assay (PGI_2). As dietary SSO will increase the linoleic acid content in the heart and linoleic acid is an inhibitor of prostaglandin synthesis by sheep vesicular microsomes (7), also the influence of linoleic acid on the release of PGI_2-like activity from the isolated rat heart was investigated.

MATERIALS AND METHODS

Animals and feeding

Two groups of male Dutch rabbits (body weight about 800 g) were fed diets (1) containing 40 per cent of the total digestable energy (en%) as fat; group 1 received 40 en% SSO (amount of dietary linoleic acid, LA: 28 en%) and group 2 37 en% L + 3 en% SSO (5 en% LA). Three groups of male Wistar rats (body weight about 250 g) were fed 35 en% SSO (25 en% LA), 33 en% L + 2 en% SSO (4 en% LA) and 30 en% HCO + 5 en% SSO (3.5 en% LA) respectively. In another experiment two groups of rats were fed 35 en% SSO or 30 en% HCO + 5 en% SSO. All groups received 23 en% soya protein (rabbits) or casein (rats) and 37 or 42 en% starch. Minerals and vitamins were supplied in sufficient amount and diets were fed ad libitum for four weeks. Besides, 3 week-old-rats were made essential-fatty-acid (EFA) deficient by feeding them diets free of linoleic acid for four months; control animals were fed a standard Muracon lab chow.

Isolated hearts

Hearts were perfused with a Krebs-Henseleit buffer according to a modified Langendorff technique (8, 9). In this modification, caval and pulmonary veins are tied off and the pulmonary artery is cannulated. Perfusate from the pulmonary artery is called Q_{rv}. Under these conditions, some fluid (Q_i) is still dripping from the apex of the heart. This fluid (Q_i) is supposed to be

interstitial effluent leaving the heart via the lymphatics. It was found (9) that Q_i contains the main quantity of the metabolites released by the heart. Q_{rv} is about 98% and Q_i about 2% of the total coronary effluent. Rabbit hearts were stimulated (10 V; duration 3 ms) at 250 and rat hearts at 360 pulses per min. Twenty min after the start of perfusion, the perfusates Q_{rv} and Q_i were collected separately for 10 or 15 min to determine the prostaglandin release.

Isolated aorta

A piece of the thoracic aorta was isolated and preperfused (5 ml min^{-1}) with a Krebs-Henseleit buffer (37 °C) for 9 min. After this period, the release of the PGI_2 was found to be constant. To increase the concentration of the PGI_2-like activity, the flow rate was lowered to 0.375 ml min^{-1} and perfusates were collected on ice for periods of 4 min.

Effect of linoleic acid on the release of PGI_2-like activity from the isolated rat heart

In the hearts of five rats fed a standard Muracon lab chow, linoleic acid was infused into the perfusion fluid. The final linoleic acid concentration was about 3 μg ml^{-1} perfusion fluid. Samples of Q_i were collected on ice for the determination of the PGI_2-like activity.

Assay of prostaglandins and PGI_2-like activity

Perfusates Q_i and Q_{rv} of the hearts of a dietary group were pooled. The prostaglandins in these perfusates were, after addition of suitable internal standards, extracted, purified by thin-layer chromatography as their methoximated pentafluorobenzyl esters and, after silylation, determined by gas chromatography with electron-capture detection (8). The determinations were singular and the results should be regarded only as semi-quantitative.

The PGI_2-like activity was determined directly in Q_i and aorta perfusates by measuring the inhibition of ADP-induced rat platelet aggregation (8); the activity was determined with respect to PGE_1. The PGI_2 values expressed in equivalents PGE_1, were divided by 22 to obtain PGI_2 in ng, as we found that in the blood platelet assay used the PGI_2 activity was 22 times that of PGE_1. PGI_2 was synthesized according to Whittaker (10) and the purity was about 80%. The release of the prostaglandins from the heart is expressed in g dry heart weight and from the aorta in cm^2.

RESULTS

The prostaglandins released from the heart into the perfusates Q_{rv} and Q_i are listed in Tables 1 and 2. From the hearts of rabbits or rats fed SSO, prostaglandin release was lower than from the hearts of animals fed HCO or L. The PGI_2-like activity in Q_i was about the same as the concentration of its

hydrolysis product 6-oxo-$PGF_{1\alpha}$, and showed similar dietary influences (Table 3). Table 4 shows the mean values of 10 determinations of the various prostaglandins released from the isolated rat heart expressed in percentages. It is evident that PGI_2 is the main prostaglandin released by the heart. These figures agree with the in-vitro results of Pace-Asciak and Rangaraj (11).

The release of PGI_2-like activity into the perfusates of isolated rat aortas is given in Table 5. The results show the same trend in the PGI_2 release as in the isolated heart.

The release of PGI_2-like activity into Q_i from hearts of EFA-deficient rats was 0.53 ± 0.13 ng $min^{-1}g^{-1}$ ($n = 11$) and from control hearts (normal rats; Muracon lab chow) 2.4 ± 0.6 ng $min^{-1}g^{-1}$ ($n = 6$). As a gentle squeezing of the heart increases prostaglandin production (12), the hearts were so treated for 15 s and Q_i was subsequently collected for 5 min. The release of PGI_2-like activity from EFA-deficient hearts and control hearts was 1.9 ± 0.3 and 6.9 ± 0.7 ng $min^{-1}g^{-1}$ respectively.

Table 6 shows that linoleic acid (3 μg ml^{-1}) has no effect on the release of the PGI_2-like activity into Q_i.

TABLE 1 Prostaglandin release (ng $min^{-1}g^{-1}$; pooled samples of 7 hearts) into the perfusates Q_i and Q_{rv} from isolated hearts of rabbits fed 40 en% sunflowerseed oil (40 SSO) or 37 en% lard + 3 en% SSO (37 L + 3 SSO) for 4 weeks.

Dietary group	Perfusate	PGE_2	$PGF_{2\alpha}$	6-oxo-$PGF_{1\alpha}$	Total
40 SSO	Q_i	1.0	0.4	2.4	5.4
	Q_{rv}	0.7	0.3	0.6	
37 L + 3 SSO	Q_i	1.7	0.7	4.2	9.6
	Q_{rv}	1.2	0.4	1.4	

TABLE 2 Prostaglandin release (ng $min^{-1}g^{-1}$; pooled samples of 7 hearts) into the perfusates Q_i and Q_{rv} from isolated hearts of rats fed 35 en% sunflowerseed oil (35 SSO) or 33 en% lard + 2 en% SSO (33 L + 2 SSO) or 30 en% hydrogenated coconut oil + 5 en% SSO (30 HCO + 5 SSO) for 4 weeks.

Expt	Dietary group	Perfusate	PGE_2	PGD_2	$PGF_{2\alpha}$	TXB_2[a)]	6-xo-$PGF_{1\alpha}$	Total
1	35 SSO	Q_i	0.6	1.0	0.3	0.3	1.5	10.2
		Q_{rv}	2.1	1.3	0.5	0.4	2.2	
	33 L + 2 SSO	Q_i	0.7	0.8	0.4	0.6	3.2	12.0
		Q_{rv}	1.5	0.6	0.4	0.6	3.2	
	30 HCO + 5 SSO	Q_i	0.6	0.6	0.4	0.7	3.9	12.9
		Q_{rv}	1.7	0.5	0.3	0.7	3.5	
2	35 SSO	Q_i	1.1	0.3	0.3	0.3	1.0	8.3
		Q_{rv}	2.0	0.6	0.5	0.2	2.0	
	30 HCO + 5 SSO	Q_i	0.6	0.3	0.4	0.2	1.1	10.0
		Q_{rv}	2.7	0.8	0.6	0.4	2.9	

a) Thromboxane B_2

TABLE 3 Release of PGI_2-like activity into Q_i from isolated hearts of rabbits fed 40 SSO or 37 L + 3 SSO and rats fed 35 SSO, 33 L + 2 SSO or 30 HCO + 5 SSO (see Tables 1 and 2) for 4 weeks.

Animal	Dietary groups	PGI_2-like activity[a)] (ng $min^{-1}g^{-1}$)
rabbit	40 SSO	2.2 ± 0.5
(n=4)	37 L + 3 SSO	3.0 ± 0.8
rat	35 SSO	1.4 ± 0.4
(n=7)	33 L + 2 SSO	1.5 ± 0.4
	30 HCO + 5 SSO	1.8 ± 0.5
rat	35 SSO	3.0 ± 0.4
(n=7)	33 L + 2 SSO	3.1 ± 0.3

TABLE 4 Release of prostaglandins from the isolated rat heart. (Calculated from Table 2; Mean of 10 determinations in 35 hearts).

Prostaglandin	Release/%
6-oxo-$PGF_{1\alpha}$	47
PGE_2	26
PGD_2	13
$PGF_{2\alpha}$	8
Thromboxane B_2	6

TABLE 5 Release of PGI_2-like activity (Mean values ± SEM; n=7) from isolated aortas of rats fed 35 en% sunflowerseed oil (35 SSO) or 30 en% hydrogenated coconut oil + 5 en% SSO (30 HCO + 5 SSO) for 4 weeks.

Dietary group	PGI_2 (ng min^{-1} cm^{-2})
35 SSO	0.37 ± 0.05
30 HCO + 5 SSO	0.49 ± 0.05

TABLE 6 Effect of linoleic acid (LA)[a] on the release of PGI_2-like activity (mean values ± SEM; n=5) into the perfusate Q_i of the isolated rat heart.

	Time (min)	PGI_2 (ng $min^{-1}g^{-1}$)
Control	20-25	2.3 ± 0.3
LA	30-35	2.6 ± 0.3
Control	40-45	2.7 ± 0.5

a) LA was infused into the perfusion fluid from 25 to 35 min after the start of the perfusion. The final concentration was 3 μg ml^{-1}, which was about twice as high as that found in the perfusate Q_i from isolated hearts of SSO fed rats.

DISCUSSION

In the present study, we could confirm and extend the results of our earlier work (8). It is now generally agreed that PGI_2 (prostacyclin) is the major prostaglandin released from isolated perfused rabbit and rat hearts (8,11,13 and 14; Tables 1-3). In perfused rat aortas we found also a significant formation of PGI_2 (Table 5). As it is not likely that these perfused preparations contain many platelets - thromboxane formation is of minor importance - we must assume that PGI_2 is synthesized from arachidonic acid in the tissues and that platelets are not the main source of the endoperoxide intermediate (15).

However, it should be realized that the experimental set-ups used were far from physiological. Another important factor is the inevitable handling, deformation and squeezing of the tissue when preparing it for the experiment. This causes a high release of PGI_2-like activity in the first phase of perfusion (unpublished results). The release decreases to become more or less constant after 15 to 20 min (8). To show that handling of the organ is a stimulus for PGI_2 production, hearts were gently squeezed, by which PGI_2 release became more than twice as much. Also in the isolated rabbit heart, squeezing increased prostaglandin release (12).

The influence of the type of dietary fat on the basal prostaglandin release from the isolated rat heart can be summarized as follows. When the diet does not contain a significant amount of linoleic acid (EFA-deficiency) there is little release of PGI_2. Increasing the amount of linoleic acid in the diet (e.g. the diets containing lard or hydrogenated coconut oil) leads to an increase in

PGI_2 production. A further increase of dietary linoleic acid (e.g. the diets containing sunflowerseed oil) does not lead to a greater PGI_2 release. Instead, the result show a slight tendency for a lower PGI_2 production.
The results are not statistically significant different. However, the observation that, under the experimental conditions, feeding diets containing more than 3.5 en% linoleic acid does not further increase the PGI_2 production in the isolated rat heart and aorta, may be of physiological significance and needs further investigation.
With respect to the release of the other prostaglandins of the 2 series (D_2, E_2, $F_{2\alpha}$, TXB_2) there seems to be no clear influences of the dietary fats (Tables 1 and 2) either. However, as the concentrations of these prostaglandins are lower than that of PGI_2, gas chromatographic determination is less accurate and any differences between the groups may not be detected.
The effect of the type of dietary fat on the basal PGI_2 production in the isolated aorta is similar to that found in the isolated heart. Supposing that the bulk of the prostaglandins released by the heart originates from the coronary vessels, the results from heart and aorta agree rather well.
Parts of our results seem to fit into those of Dupont et al. (16) who found that PGE_2 production rate of blood platelets did not increase in groups of rats when fed increasing amounts of linoleate (from 5 to 30 en%), although there was a linear increase in PGE_1 and $PGF_{2\alpha}$ production rate. The latter results are about the same as those obtained by Hwang et al. (17) who found that after feeding corn oil the serum PGE_1 and $PGF_{2\alpha}$ concentrations were higher than after feeding beef fat.
Linoleic acid inhibits prostaglandin biosynthesis in sheep vesicular gland microsomes (unpublished results, see also Ref. 7), but we found that in the isolated heart, perfused with a perfusion fluid containing about the same amount of linoleic acid as found in the perfusates of the hearts of the sunflowerseed oil groups, linoleic acid did not influence PGI_2 release (Table 6). Therefore, it is unlikely that the possible differences between the PGI_2 releases of the dietary groups can be explained by the inhibiting activity of free linoleic acid.
It is clear that the type of dietary fat can influence the synthesis of prostaglandins, but it is not yet possible to give a more quantitative appreciation of the phenomenon observed.

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ADDENDUM VI

Short communication

EFFECTS OF PROSTAGLANDINS ON CORONARY FLOW RATE AND LEFT VENTRICULAR WORK IN ISOLATED RAT HEART*

EMILE A.M. DE DECKERE

Unilever Research Vlaardingen, The Netherlands

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In isolated rat hearts PGD_2, PGE_1, PGE_2, $PGF_{2\alpha}$ and PGI_2 increased coronary flow rate at concentrations of about 23, 1.4, 0.4, 2.5 and 0.6 nmol per l respectively. PGD_2, PGE_2 and PGI_2 did not affect left ventricular work (W_{1v}). PGE_1 (14 nmol/l) lowered W_{1v} (12%) and $PGF_{2\alpha}$ (9 nmol/l) increased W_{1v} (10%). It is concluded that endogenously released PGI_2 can probably affect coronary flow rate.

Prostaglandins Isolated rat heart Coronary flow rate Left ventricular work

1. Introduction

The prostaglandins (PG) D_2, E_2, $F_{2\alpha}$ and I_2 are released by the isolated rat heart (De Deckere et al., 1977). Of these prostaglandins, PGE_2 increases the coronary flow rate in the isolated rat heart (Vergroesen et al., 1967), whereas $PGF_{2\alpha}$ has no effect (Vergroesen et al., 1969). No data have been published about the effects of the prostaglandins on left ventricular work in the isolated working rat heart.

As the effects of the prostaglandins released by the heart on the isolated rat heart have been insufficiently described, we studied the effects of PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and PGE_1 on coronary flow rate and left ventricular work of the isolated rat heart. The minimal amounts of prostaglandins needed to obtain an effect were estimated in order to compare with the amount of prostaglandins formed to be released by the isolated heart.

2. Materials and methods

Isolated hearts of rats (body mass about 300 g) were perfused with an oxygenated (95 O_2/5 CO_2) Krebs-Henseleit buffer (37.5°C) supplemented with 11.1 mmol per l glucose and 0.5 mmol per l EDTA. Perfusion pressure was 9.3 kPa; stimulation frequency 360 min^{-1}. Experiments were started 15 min after the start of perfusion.

The effects of PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and PGE_1 on coronary flow rate (Q_{cor}) were tested in the Langendorff perfused heart. $\dot{Q}_{cor}$ (ml/min/g dry heart mass) was determined by collecting the coronary effluent every minute. Solutions of the prostaglandins (7.2×10^{-8} to 1.4×10^{-6} mol per l) were infused (0.02 to 0.2 ml/min) into the perfusion fluid just above the heart.

The effect of the prostaglandins on left ventricular work (W_{1v}) was tested in the working rat heart preparation (De Deckere and Ten Hoor, 1979). W_{1v} (J/min/g dry heart mass) at a mean atrial pressure of 1.33 kPa, was calculated from the output of the left ventricle and the mean systolic aortic pressure.

Solutions of the prostaglandins (5.7×10^{-7} to 2.9×10^{-5} mol/l) were infused (0.2—0.5 ml per min) into the perfusion fluid just before entering the left atrium. W_{1v} and $\dot{Q}_{cor}$ were estimated just before prostaglandin infusion, after 4 min of prostaglandin infusion and 5 min after termination of prostaglandin infusion.

PGD_2, PGE_1, PGE_2 and $PGF_{2\alpha}$ were stored in methanol at −20° C. For infusion, these prostaglandins were dissolved in Krebs-Henseleit buffer after evaporating the methanol with nitrogen. PGI_2 was stored in 0.1 ml/l NaOH at 0°C. To keep PGI_2 stable, infusion solutions of PGI_2 were made in 1.3 mmol/l NaOH. Infusion of this NaOH solution had no effect on the pH of the perfusion fluid.

To obtain a dry heart mass, hearts were dried at 80°C for 24 h. Student's *t*-test for paired data was used for statistical analyses.

3. Results

All the prostaglandins tested increased the coronary flow rate in the Langendorff perfused rat heart. Table 1 shows the highest concentrations which did not affect $\dot{Q}_{cor}$ and the lowest concentrations at which $\dot{Q}_{cor}$ started to increase significantly. Higher concentrations of PGE_1, PGE_2 and PGI_2 gave higher values of $\dot{Q}_{cor}$: the maximal values of $\dot{Q}_{cor}$ obtained were between 100 and 120 ml/min/g. At these higher concentrations, $\dot{Q}_{cor}$ started to increase within one min (see PGI_2, table 1). Much higher concentrations of $PGF_{2\alpha}$ did not further increase $\dot{Q}_{cor}$. Moreover, $PGF_{2\alpha}$ — even at very high concentrations — increased $\dot{Q}_{cor}$ only after a lapse of about one min. PGD_2 increased $\dot{Q}_{cor}$ only at concentrations higher than 23 nmol/l.

PGD_2 (n = 3), PGE_2 (n = 5) and PGI_2 (n = 5) did not affect W_{1v} at concentrations of 143, 74 and 34 nmol/l respectively. $PGF_{2\alpha}$ at a concentration of 8.6 nmol/l increased W_{1v} from 6.4 ± 0.3 to 7.0 ± 0.03 J/min/g (n = 3; $P_2 < 0.02$); W_{1v} increased further to 7.2 ± 0.3 J/min/g when the $PGF_{2\alpha}$ concentration was doubled. At 72 nmol/l, W_{1v} increased from 6.1 ± 0.6 to 7.4 ± 0.6 J/min/g (n = 3; $P_2 < 0.05$). PGE_1 at a concentration of 14 nmol/l decreased W_{1v} from 5.9 ± 0.2 to 5.2 ± 0.2 J per min per g (n = 6; $P_2 < 0.01$); W_{1v} decreased further to 4.8 ± 0.2 J/min/g when the PGE_1 concentration was increased to 37 nmol/l. In all working rat hearts, $\dot{Q}_{cor}$

TABLE 1

Effect of prostaglandins E_1, E_2, $F_{2\alpha}$ and I_2 (nmol/l) [1] on coronary flow rate ($\dot{Q}_{cor}$, ml/min/g dry heart mass ± S.E.M.) of Langendorff perfused rat hearts. Prostaglandin infusion was started at t = 0.

t (min)	PGE_1		PGE_2		$PGF_{2\alpha}$			PGI_2			
	0.7 n = 4	1.4 n = 6	0.2 n = 3	0.4 n = 7	1.7 n = 3	2.5 n = 7	580.0 n = 4	0.3 n = 3	0.6 n = 4	1.1 n = 6	3.7 n = 3
0 [2]	69 ± 3	56 ± 3	66 ± 6	64 ± 4	65 ± 4	58 ± 3	56 ± 4	54 ± 4	59 ± 6	58 ± 4	54 ± 3
1	69 ± 5	63 ± 3	66 ± 6	67 ± 4	66 ± 4	58 ± 3	53 ± 2	53 ± 4	62 ± 7	65 ± 5	75 ± 2
2	73 ± 5	71 ± 2	66 ± 6	70 ± 4	66 ± 4	64 ± 4	71 ± 3	55 ± 4	69 ± 7	81 ± 5	97 ± 2
3	72 ± 5	75 ± 3	67 ± 6	74 ± 4	66 ± 4	72 ± 4	89 ± 2	56 ± 4	72 ± 7	90 ± 4	107 ± 1
4	72 ± 5	78 ± 3	67 ± 6	76 ± 4	66 ± 4	83 ± 3	89 ± 2	56 ± 4	76 ± 7	93 ± 4	110 ± 1
t = 4 vs. t = 0	$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.001$	$P < 0.01$		$P < 0.001$	$P < 0.001$

[1] Final concentration in the perfusion fluid at t = 0. When $\dot{Q}_{cor}$ increased during prostaglandin infusion, the final concentration decreased proportionally.
[2] $\dot{Q}_{cor}$ was constant for 5 min before prostaglandin infusion.

was increased by the prostaglandins. As $\dot{Q}_{cor}$ was already rather high (95—107 ml/min/g) in the working heart, the increase was small (< 20%).

4. Discussion

In the isolated perfused rat heart at constant perfusion pressure, the prostaglandins E_1, E_2, $F_{2\alpha}$ and I_2 increased coronary flow rate at low concentrations. PGE_2 and PGI_2 were the most potent substances; they were about equipotent. These results agree with data obtained for the isolated guinea pig heart (Krebs and Schrör, 1975) and for the dog heart (Kaley et al., 1977). The observed decrease in coronary resistance by $PGF_{2\alpha}$ is in contrast with the results obtained by Vergroesen et al. (1969) for the rat heart and with those by Hollenberg et al. (1968) for the dog heart.

The fact that the increase in coronary flow rate by $PGF_{2\alpha}$ was delayed for about one min suggests that the effect of $PGF_{2\alpha}$ was indirect. We recently found that in the isolated rat heart, $PGF_{2\alpha}$ doubled the release of PGE_2 and PGI_2 (De Deckere et al., to be published). Murota et al. (1978) found that in cultured fibroblasts, $PGF_{2\alpha}$ doubled the release of arachidonic acid. So the increase of coronary flow rate by $PGF_{2\alpha}$ can be explained by its stimulation of PGE_2 and PGI_2 synthesis in the heart.

In the working rat heart, PGD_2, PGE_2 and PGI_2 did not affect left ventricular work, whereas PGE_1 decreased it and $PGF_{2\alpha}$ increased it. Whether prostaglandins have a positive or negative inotropic effect can depend on the kind of heart preparation used. The working rat heart was chosen for the present study as this preparation is close to the in vivo situation.

From a previous study (De Deckere et al., 1977), the PGE_2 and PGI_2 concentrations in the total effluent from the isolated rat heart can be calculated to be about 0.13 and 0.23 nmol/l respectively. These concentrations do not differ much from those of PGE_2 and PGI_2 which elicit vasodilation. So we may conclude that the concentrations of endogeneous prostaglandins — especially after stimulation of synthesis — are high enough to affect the coronary flow rate in the isolated rat heart.

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ADDENDUM VII

THE ROLE OF PROSTAGLANDINS IN THE REGULATION OF THE SPONTANEOUS FREQUENCY, CORONARY FLOW RATE AND LEFT VENTRICULAR WORK IN THE ISOLATED RAT HEART. SOME COMPARISONS WITH THE ISOLATED GUINEA PIG HEART.*

Running title: Role of prostaglandins in rat heart

By E.A.M. de Deckere and F. ten Hoor
(Unilever Research Vlaardingen, The Netherlands)

Summary

In the isolated perfused rat heart the role of prostaglandins (PG) in the regulation of spontaneous frequency (f), coronary flow rate ($\dot{Q}_{cor}$) and maximal external left ventricular work (W_{lv}(max)) was studied by inhibiting the PG-synthesis in several ways. Partial inhibition of PG-synthesis by feeding acetylsalicyclic acid (ASA) for a few days or linseed oil for 4 weeks did not affect $\dot{Q}_{cor}$ and W_{lv} (max); f was decreased by ASA but not by linseed oil. Complete or almost complete inhibition of PG-synthesis by i.p. injection of ASA or indomethacin or by perfusion with indomethacin decreased $\dot{Q}_{cor}$, but f and W_{lv}(max) were not affected. In the isolated guinea pig heart PGI_2-release was very low compared to that from the rat heart. Perfusion with arachidonic acid, indomethacin or $PGF_{2\alpha}$ affected $\dot{Q}_{cor}$ immediately, whereas in the rat heart it took at least one min before $\dot{Q}_{cor}$ changed.

It is concluded that in the isolated rat heart prostaglandins are involved in the regulation of coronary flow rate, but not in that of left ventricular work. The different effects on spontaneous frequency after feeding and injecting ASA suggest that prostaglandins are indirectly involved in the regulation of heart frequency.

Introduction

Feeding sunflowerseed oil to rats compared to feeding saturated fats increases coronary flow rate and external left ventricular work in their isolated hearts (3). Prostaglandins (PGs) might participate in causing these effects, as

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linoleic acid which is present in a large amount in sunflowerseed oil is the ultimate precursor of prostaglandins of the 1- and 2-series, and as feeding linoleic acid-rich diets increases PG-synthesis in vivo (5). It is well known that PGs are released from isolated heart preparations and that exogenously supplied PGs or inhibition of PG-synthesis can affect coronary flow rate and myocardial contractile force, but it is still very doubtful whether PGs participate in regulating coronary flow rate and myocardial force under physiological conditions (1). To determine whether in the isolated heart PGs participate in regulating spontaneous frequency (f), coronary flow rate ($\dot{Q}_{cor}$) and external left ventricular work (W_{lv}), PG-synthesis was partly or completely inhibited in several ways to establish effects on f, $\dot{Q}_{cor}$ and W_{lv}.

Materials and methods

Use was made of male Wistar rats and guinea pigs weighing 250 to 450 g and about 700 g respectively just before isolation of the heart. The animals had free access to food and water, and were fed commercially available laboratory chows or, where mentioned, semisynthetic diets (for 4 w) which contained 5, 35 or 50 en% fat, casein (23 en%), starch and sufficient amounts of vitamins and minerals. The fats used were sunflowerseed oil (SSO; 70% 18 : 2 (n-6)), linseed oil (LSO; 16% 18:2 (n-6), 52% 18:3 (n-3)), lard (L; 9% 18:2 (n-6)) and hydrogenated coconut oil (HCO; 0% 18:2 (n-6)). Three-week-old rats were made essential fatty acid (EFA) deficient by feeding them an EFA-deficient diet containing 5 en% HCO for 3 months. In a number of experiments acetylsalicylic acid (ASA) was added to the food for 2, 3 or 5 days. Rats received about 194 μmol ASA per day. ASA did not influence food consumption.

For intraperitoneal (i.p.) injection of ASA and indomethacin, ASA was suspended and partly dissolved in 1.5 ml saline after which an equimolar amount of NaOH was added. The pH remained below 7 and the solution was injected immediately. Indomethacin was dissolved in 0.2 ml ethanol, after which 1.5 ml Krebs-Henseleit buffer was added. Eighty three μmol ASA and 28 μmol indomethacin per kg body mass were injected 1.5 h before isolation of the heart. Control rats received saline or Krebs-Henseleit buffer. The animals were anaesthetized (sodium pentobarbital 60 mg kg^{-1}) and decapitated. Hearts were excised and perfused immediately (t = 0 min) with a Krebs-Henseleit buffer supplemented with 11.1 mmol l^{-1} glucose and 0.5 mmol l^{-1} EDTA. The perfusion fluid (38 °C) was gassed with 95% O_2 + 5% CO_2. In the Langendorff-perfused rat heart perfusion pressure (P_p) was 9.3 or 10.6 kPa and in the guinea pig heart 9.3 kPa (1 kPa = 7.5 mm Hg). In the working heart (3) perfusion was done via the left atrium at left ventricular filling pressures (P_{lvf}) of 0.80, 1.06, 1.33, 1.46 and 1.60 kPa; the hydrostatic aortic pressure was 9.3 kPa. External work of the left ventricle (W_{lv}; J $min^{-1}g^{-1}$ dry heart mass) was calculated from the output of the left ventricle and the mean systolic aortic

pressure. The maximal value of W_{lv}(W_{lv}(max)) was obtained at P_{lvf} = 1.33 kPa. Spontaneous frequency (f) was determined at t = 10 min by counting the pressure pulsations in the aortic cannula. Pressure was measured using a Statham P23Db pressure transducer. Hearts were stimulated from t = 3 min on, or after the determination of f, at a frequency of 360 min^{-1} (rat) and 300 min^{-1} (guinea pig) (10 V, duration 3 ms), except where mentioned otherwise. Coronary flow rate ($\dot{Q}_{cor}$; ml min^{-1} dry heart mass was measured at t = 10 to 25 min (except where mentioned otherwise) by collecting coronary effluent during 1 min periods.

The isolated perfused rat aorta was perfused with a Krebs-Henseleit buffer as described previously (2).

For perfusions with indomethacin, arachidonic acid (AA) and $PGF_{2\alpha}$, indomethacin was dissolved in ethanol (55 μmol ml^{-1}) and diluted with Krebs-Henseleit buffer to concentrations between 0.35 and 4.2 μmol ml^{-1}, AA (0.17 μmol ml^{-1}) in saline under N_2 to which NaOH (1.5 μmol ml^{-1}) was added and $PGF_{2\alpha}$ (5.7 nmol ml^{-1}) in Krebs-Henseleit buffer. The compounds were infused into the perfusion fluid just above the heart. Infusion of the vehicles into the perfusion fluid had no effect on Q_{cor} and W_{lv}.

PGI_2-like activity of perfusate was determined by measuring the inhibitory activity of a sample of the perfusate on ADP-induced rat platelet aggregation (2). Heparin (500 IU l^{-1}) was added to the perfusion fluid to prevent clotting of the platelets into the bioassay. In an aggregometer (Chrono-Log corporation) 0.35 ml test sample, 0.05 ml platelet rich plasma and 0.05 ml of a PGE_1 solution (57 pmol ml^{-1}) or of a PGI_2 solution (8 pmol ml^{-1}) were incubated for 1 min, after which 0.05 ml of an ADP solution (3.3 nmol ml^{-1}) was added. The inhibition by test samples was compared with inhibition by PGI_2. The PGI_2-like substance of test samples is denoted as PGI_2 (criterion: inhibition of ADP-induced aggregation which disappeared after 20 min of standing of the samples at room temperature). PGI_2-release was determined from t = 15 to 25 min, except where mentioned otherwise. Statistical analysis was performed using Student's t test for paired and unpaired data or Newman Keuls multiple range test.

Results

In the Langendorff perfused heart, feeding ASA for 2 and 5 days to groups (n = 6) of rats fed a commercial diet or a semisynthetic diet containing 50 en% SSO significantly decreased PGI_2-release by about 50% and f by about 20% (control f: 330 min^{-1}); $\dot{Q}_{cor}$ was not affected. In hearts of EFA-deficient rats, feeding ASA for 3 days did not affect f [305 ± 18 (ASA) vs 303 ± 10 (control) min^{-1}; n = 8], and increased $\dot{Q}_{cor}$ from 66 ± 3 (control), to 83 ± 4 ml min^{-1} g^{-1} ($P_2 < 0.01$; P_p = 10.6 kPa). In the working heart, feeding ASA for 3 days did not affect W_{lv}. In the isolated perfused aorta, feeding ASA for 2 days decreased

PGI_2 release from 6.0 ± 1.8 (control) to 3.3 ± 0.6 pmol $min^{-1}cm^{-2}$ (n = 4). After feeding groups (n = 7) of rats semisynthetic diets containing 35 en% SSO or LSO, the release of PGI_2 in the Langendorff perfused rat heart was 38 ± 11 and 12 ± 5 pmol $min^{-1}g^{-1}$ respectively ($P_2 < 0.05$); f and $\dot{Q}_{cor}$ were not affected. The values of f, W_{lv}(max) and PG-release in the working heart after feeding diets containing 35 en% LSO, SSO or L are shown in Table 1. Feeding these diets did not result in differences in the mean body and dry heart masses of the groups.

Table 1. Mean values (± SEM; n = 9) of spontaneous frequency (f), external left ventricular work (W_{lv}) and prostaglandin release (determined in pooled coronary effluents of 6 hearts) of isolated working hearts of rats fed 35 en% linseed oil (LSO), sunflowerseed oil (SSO) or lard (L) for 4 weeks. f was determined at a left ventricular filling pressure (P_{lvf}) of 0.80 kPa; hearts were then stimulated at a frequency of 360 min^{-1}. For the determination of the release of prostaglandins coronary effluent was collected during 1 min periods at P_{lvf} = 0.80, 1.06, 1.33 and 1.60 kPa.

Dietary group	f (min^{-1})	W_{lv}(max)[1] ($J\,min^{-1}\,g^{-1}$)	PGE_2	PGF_2	6-keto-PGF	Total
			(pmol $min^{-1}\,g^{-1}$)			
LSO	324 ± 5	5.68 ± 0.24	5	2	17	24
SSO	330 ± 8	6.16 ± 0.24	13	3	32	48
L	304 ± 5	5.58 ± 0.22	11	2	34	47

[1]) P_{lvf} = 1.33 kPa.

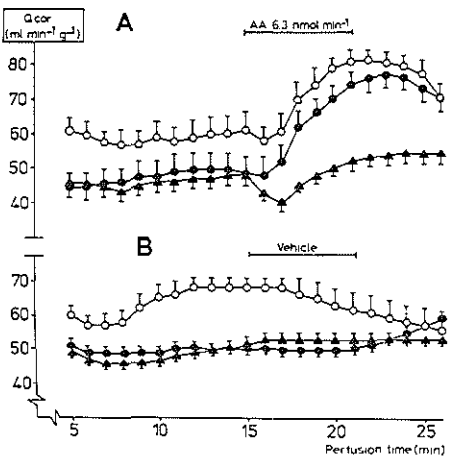

Fig. 1. Influence of arachidonic acid (AA) (Fig. 1A) or its vehicle (Fig. 1B) on coronary flow rate ($\dot{Q}_{cor}$) of isolated hearts of rats after i.p. injection of saline (o——o), ASA (83 μmol kg^{-1}; ▲——▲) or indomethacin (28 μmol kg^{-1}; ●——●) 1.5 h before heart isolation. AA was infused into the perfusion fluid above the heart resulting in a mean concentration of 0.43 (saline-group), 0.54 (ASA-group) and 0.50 (inomethacin-group) nmol ml^{-1} on t = 15 min. Perfusion pressure 9.3 kPa. Pacing frequency 360 min^{-1}. Mean values ± SEM; n = 6. On t = 15 min: $\dot{Q}_{cor}$ (saline) > $\dot{Q}_{cor}$ (ASA) = $\dot{Q}_{cor}$ (indomethacin); P < 0.05.

In the Langendorff perfused rat heart ASA (i.p.) completely inhibited PGI_2-release throughout perfusion time. Indomethacin (i.p.) completely inhibited PGI_2-release during the first min of perfusion. PGI_2-release was very low until about 20 min after starting the perfusion; from that time on it increased (Table 2). ASA and indomethacin slightly decreased f (by about 5%) and significantly decreased $\dot{Q}_{cor}$; in the indomethacin group $\dot{Q}_{cor}$ starts to increase after about 20 min of perfusion (Fig. 1). In control hearts a weak correlation was found between PGI_2-release and $\dot{Q}_{cor}$ [$\dot{Q}_{cor} = 0.23 \cdot$ (PGI_2-release) + 57; r = 0.43; n = 12]. Perfusion with AA increased PGi_2 release and $\dot{Q}_{cor}$ (Fig. 1) in the control and indomethacin group; in the ASA group no PGI_2-release was found and $\dot{Q}_{cor}$ decreased and then slightly increased. In the isolated working rat heart i.p. injection of ASA did not affect W_{lv} [W_{lv}(max): 6.27 ± 0.17 (ASA) vs 6.25 ± 0.19 (control) J $min^{-1}g^{-1}$; n = 6].

Table 2. Release of PGI_2 (pmol $min^{-1}g^{-1}$; mean values ± SEM) from isolated perfused hearts of rats after i.p. injection of saline, indomethacin (28 μmol kg^{-1}) and ASA (83 μmol kg^{-1}) 1.5 h before isolation of the heart

Perfusion time (min)	AA perfusion[1])	Group				
		Saline		Indomethacin		ASA
2	—	123 ± 27	(n = 3)	2	(n = 3)	2 (n = 3)
10-15	—	33 ± 6	(n = 12)	4 ± 1	(n = 9)	2 (n = 6)
19	+	198 ± 39	(n = 7)	84 ± 15	(n = 6)	2 (n = 4)
30	—	18	(n = 2)[2])	45	(n = 2)[2])	2 (n = 2)[2])

[1]) Arachidonic acid (AA; 6.3 nmol min^{-1}) was infused from t = 15 to 22 min (see Fig. 1) resulting in a concentration at the start of the infusion of about 0.5 nmol ml^{-1}.
[2]) These hearts were not perfused with AA (Fig. 1B).
Perfusion pressure 9.3 kPa. Pacing frequency 360 min^{-1}.

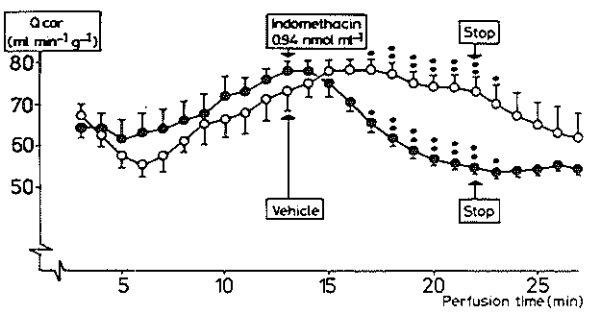

Fig. 2. Effect of indomethacin on coronary flow rate ($\dot{Q}_{cor}$) in the Langendorff perfused rat heart. Indomethacin or Krebs-Henseleit buffer (vehicle) was infused into the perfusion fluid above the heart. The indomethacin concentration at the start of the infusion is presented. P_p = 9.3 kPa. Pacing frequency 360 min^{-1}. Mean values ± SEM; n = 6. * $P_2 < 0.01$; ** $P_2 < 0.002$.

In the isolated rat heart, perfusion with indomethacin (about 0.9 nmol ml^{-1}) completely inhibited PG-synthesis from endogenously and exogenously supplied AA (0.4 nmol ml^{-1}) and significantly decreased $\dot{Q}_{cor}$ (Fig. 2); after stopping indomethacin infusion $\dot{Q}_{cor}$ starts to increase after about 7 min. Higher concentrations of indomethacin (about 2.5 nmol ml^{-1}) did not affect $\dot{Q}_{cor}$ and still higher concentrations (>5.5 nmol ml^{-1}) increased $\dot{Q}_{cor}$. During indomethacin perfusion, AA first decrease and then slightly increased $\dot{Q}_{cor}$.

In the isolated guinea pig heart (n = 9) $\dot{Q}_{cor}$ decreased gradually after the start of perfusion from 44 ± 3 (t = 3 min) to 30 ± 1 ml $min^{-1}g^{-1}$ (t = 15 min) after which $\dot{Q}_{cor}$ remained constant. Release of PGI_2 on t = 2 min was 16 ± 5 and on t = 18 min <2 pmol $min^{-1}g^{-1}$. Perfusion with AA (about 0.3 nmol ml^{-1} at the start of infusion) increased $\dot{Q}_{cor}$ by about 70% and PGI_2-release to 157 ± 5 pmol $min^{-1}g^{-1}$ (n = 3), being about 2.4% of the infused AA. Perfusion with indomethacin (about 0.4 nmol ml^{-1}) completely inhibited PGI_2-synthesis and decreased $\dot{Q}_{cor}$ by about 15% (n = 3). During indomethacin perfusion, AA did not affect $\dot{Q}_{cor}$. After stopping indomethacin perfusion $\dot{Q}_{cor}$ gradually increased within 7 min to its original value. The AA-stimulated PGI_2-release was still partly inhibited 30 min after stopping indomethacin perfusion.
Perfusion with $PGF_{2\alpha}$ (19 pmol ml^{-1}) increased $\dot{Q}_{cor}$ by about 12% and PGI_2-release to 42 pmol $min^{-1}g^{-1}$. The changes in $\dot{Q}_{cor}$ and PGI_2-release brought about by perfusion with AA, indomethacin or $PGF_{2\alpha}$ begin immediately after the start of the infusions.

Discussion

In the isolated heart of normal rats, complete inhibition of PG-synthesis by i.p. injection of ASA or indomethacin and by perfusion with indomethacin decreased coronary flow rate ($\dot{Q}_{cor}$). Also in the isolated guinea pig heart, as found by Schrör *et al.* (6), $\dot{Q}_{cor}$ was decreased by perfusion with indomethacin. In the rat and guinea pig heart perfusion with arachidonic acid (AA) increased both PGI_2-release and $\dot{Q}_{cor}$, but when PGI_2-synthesis was completely inhibited by indomethacin (perfusion) or ASA (i.p., rat only) $\dot{Q}_{cor}$ decreased, and then slightly increased (rat heart), or there was no effect (guinea pig heart). These results indicate that endogenously synthetized PGs affect $\dot{Q}_{cor}$ in the isolated perfused rat and guinea pig heart. However, in the rat heart, partial inhibition of PG-synthesis by feeding ASA or linseed oil did not affect $\dot{Q}_{cor}$ and in the guinea pig heart, $\dot{Q}_{cor}$ starts to increase immediately at the end of the indomethacin perfusion, whereas the AA stimulated PGI_2-synthesis is still partly inhibited 30 min after the indomethacin perfusion. From these results it may be concluded that only a part of the synthetized PGs affects $\dot{Q}_{cor}$. The fact that after perfusion with indomethacin (or after i.p. injection) PG-synthesis is still inhibited for some time indicates that indometh-

acin could be bound to the cyclo-oxygenase rather tightly. EFA-deficiency decreases $\dot{Q}_{cor}$ (7) and PGI_2-release (2) in the isolated rat heart. Feeding ASA to EFA-deficient rats increased $\dot{Q}_{cor}$ which shows that ASA under certain conditions can also increase $\dot{Q}_{cor}$. There are some differences between the rat and guinea pig heart. In the isolated guinea pig heart $\dot{Q}_{cor}$ is very stable and PGI_2-release is low (8) compared to the rat heart which might indicate that $\dot{Q}_{cor}$ and PG-synthesis are less disturbed by the perfusion conditions. In the guinea pig heart, AA, indomethacin and $PGF_{2\alpha}$ affect $\dot{Q}_{cor}$ (and PGI_2-synthesis) immediately, whereas in the rat heart, it takes at least one min before $\dot{Q}_{cor}$ is affected. Therefore, the guinea pig heart seems better suitable for a further study of the participation of PGs in the regulation of $\dot{Q}_{cor}$ than the rat heart. It is possible that PGs are involved in the regulation of the spontaneous frequency (f). Feeding ASA decreased PG-synthesis and f in normal hearts but did not affect f in EFA-deficient hearts. In the latter hearts, PG-synthesis and f have already been lowered compared to the values in normal hearts. However, i.p. injection of ASA and indomethacin inhibited PG-synthesis completely but f only slightly. As PGs are involved in the noradrenaline release in the heart (4) it is possible that by the partial inhibition of PG-synthesis for a few days by feeding ASA the mechanism which determines heart frequency is affected.

It is concluded that in the isolated rat heart endogenously formed prostaglandins affect coronary flow rate. Prostaglandins may also affect spontaneous frequency probably by influencing another mechanism, e.g. neurotransmitter release. We found no indications that external left ventricular work is affected by endogenously synthetized prostaglandins.

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First author's adress:
E.A.M. de Deckere, Unilever Research Vlaardingen, P.O. Box 114, 3130 AC Vlaardingen, The Netherlands.

CURRICULUM VITAE

Op 15 mei 1942 werd ik geboren te Hulst waar ik ook in 1961 het diploma H.B.S.-B behaalde. Na het vervullen van de militaire dienstplicht ging ik in 1963 biologie studeren in Leiden. Hierin studeerde ik in 1969 af met als hoofdvak biochemie. Vanaf 1 april 1969 ben ik werkzaam op het Unilever Research Laboratorium te Vlaardingen, tot juni 1971 op de afdeling Microbiologie en vervolgens, tot op heden, in de sectie Pathofysiologie.