Properties of phosphatidate phosphohydrolase and diacylglycerol acyltransferase activities in the isolated rat heart

Effect of glucagon, ischaemia and diabetes

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Myocardial triacylglycerol hydrolysis is subject to product inhibition. After hydrolysis of endogenous triacylglycerols, the main proportion of the liberated fatty acids is re-esterified to triacylglycerol, indicating the importance of fatty acid re-esterification in the regulation of myocardial triacylglycerol homeostasis. Therefore, we characterized phosphatidate phosphohydrolase (PAP) and diacylglycerol acyltransferase (DGAT) activities, enzymes catalysing the final steps in the re-esterification of fatty acids to triacylglycerols in the isolated rat heart. The PAP activity was mainly recovered in the microsomal and soluble cell fractions, with an apparent K_m of 0.14 mM for both the microsomal and the soluble enzyme. PAP was stimulated by Mg^{2+} and oleic acid. Oleic acid, like a high concentration of KCl, stimulated the translocation of PAP activity from the soluble to the particulate (microsomal) fraction. Myocardial DGAT had an apparent K_m of 3.8 μ M and was predominantly recovered in the particulate (microsomal) fraction. Both enzyme activities were significantly increased after acute streptozotocin-induced diabetes, PAP from 15.6 ± 1.1 to 28.1 ± 3.6 m-units/g wet wt. ($P < 0.01$) and DGAT from 2.23 ± 0.11 to 3.01 ± 0.11 m-units/g wet wt. ($P < 0.01$). In contrast with diabetes, low-flow ischaemia during 30 min did not affect PAP and DGAT activity in rat hearts. Perfusion with glucagon (0.1 μ M) during 30 min did not affect total PAP activity, but changed the subcellular distribution. More PAP activity was recovered in the particulate fraction. DGAT activity was lowered by glucagon treatment from 0.37 ± 0.03 to 0.23 ± 0.02 m-unit/mg of microsomal protein ($P < 0.05$). The role of PAP and DGAT activity and PAP distribution in the myocardial glucose/fatty acid cycle is discussed.

INTRODUCTION

In the normoxic heart, fatty acids are the main source of energy, 60–70 % of the overall energy demand being covered by β -oxidation of fatty acids [1,2]. Recent experiments suggest the importance of a glucose/fatty acid cycle in the regulation of triacylglycerol metabolism [3,4]. Such a cycle was proposed to protect the heart against the potential harmful effects of high intracellular concentrations of non-esterified fatty acids [5–7].

Several lipases have been described to play a role in the degradation of endogenous myocardial triacylglycerols; intracellular lipoprotein lipase [8], hormone-sensitive lipase [9–11] and the lysosomal acid lipase [12–14]. The main determinant of all these lipase activities is feedback inhibition by product fatty acids [15–17]. A large portion of fatty acids released after lipase activity in the myocardium, however, is re-esterified, mainly to triacylglycerol [18,19]. Therefore an increased rate of re-esterification could attenuate the product inhibition by fatty acids. An increased rate of fatty acid re-esterification as well as degradation of triacylglycerol have been demonstrated under various conditions such as myocardial ischaemia [5,20,21], diabetes [22–24] and perfusion of isolated hearts with adrenaline, isoprenaline or lactate [5,25,26].

In contrast with the regulation of myocardial triacylglycerol degradation, only limited information is available about the regulation of myocardial triacylglycerol synthesis [27,28]. In liver and adipose tissue, phosphatidate phosphohydrolase (PAP), the enzyme that catalyses the hydrolysis of phosphatidic acid to diacylglycerol, and diacylglycerol acyltransferase (DGAT), which catalyses the final re-esterification step of diacylglycerol to triacylglycerol, have been suggested to play an important regu-

latory role in triacylglycerol synthesis [29,30]. Triacylglycerol synthesis is believed to take place in the reticular membrane system, based on the localization of the final enzyme, DGAT, at the cytosolic surface of the endoplasmic reticulum [27]. PAP, on the other hand, is localized in various compartments, and is enriched in the cytosolic and microsomal fractions [27,29]. The stimulation of PAP activity by fatty acids in liver probably occurs by a translocation of the free enzyme from the cytosol to the microsomes, where it is bound and where triacylglycerol synthesis subsequently takes place [29]. Regulation of both enzymes may occur by a phosphorylation/dephosphorylation mechanism [27], and effects of fatty acids on both enzyme activities in liver have been described [29,30]. Because of the important role of both enzymes in triacylglycerol synthesis, we characterized both enzymes in the isolated rat heart and we studied the effect of glucagon, diabetes, myocardial ischaemia and fatty acids on both enzyme activities and on the translocation of PAP from the cytosol to the microsomes.

MATERIALS AND METHODS

Animals and animal treatments

Male fed rats of the Rp strain (200–250 g body wt.) were used throughout the study. They were housed under controlled conditions (temp. 20–23 °C, lights on 07:00–19:00 h). The rats had free access to standard laboratory chow and tap water until the beginning of the experiment. Diabetes was induced by intravenous (tail-vein) injection of streptozotocin (110 mg/kg body wt., freshly dissolved in 50 mM-citrate buffer, pH 4.5)

Abbreviations used: PAP, phosphatidate phosphohydrolase; DGAT, diacylglycerol acyltransferase.

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2 days before rats were killed. The serum glucose increased from 12.1 ± 1.0 to 35.3 ± 1.8 mM at the moment of killing.

Perfusion protocol

From diethyl-ether-anaesthetized rats, the hearts were quickly excised and perfused retrogradely in an open system according to Langendorff at a constant pressure of 80 cmH₂O to remove the contained blood, as described elsewhere [31]. In a number of experiments low-flow ischaemia was induced by decreasing the coronary flow to 1 ml/min during 30 min, exactly as described elsewhere [5]. At the end of the perfusion, the hearts were removed from the perfusion apparatus, minced fine and homogenized (10%, w/v) in ice-cold 0.25 M-sucrose/1 mM-EDTA/1 mM-Tris/HCl, pH 7.4, by using an Ultra-Turrax blender (Polytron, two 15 s bursts at setting 5), followed by homogenization in a Teflon/glass Potter-Elvehjem homogenizer (ten up-and-down strokes of a tightly fitting pestle, slowly rotating). Subcellular fractions were obtained as described in ref. [31]. Pellets of the subcellular fractions obtained were suspended in 0.25 M-sucrose/1 mM-EDTA/1 mM-Tris/HCl, pH 7.4, and stored at -80°C until use. The purification of the final fractions was estimated by using marker enzymes as described in [31]. The results are expressed in accordance with the scheme of De Duve [32] for tissue fractionation studies. The relative specific activity (RSA) is defined as percentage of total PAP activity within each fraction/percentage of total protein. The contamination of the lysosomal fraction with microsomes, as judged by measurement of rotenone-insensitive NADPH-cytochrome *c* reductase, was 25%. There was almost no contamination of the particulate fractions with the cytosolic fraction.

Analytical procedures

PAP activity was measured essentially as described in [33]. Phosphatidate (1.2 mM) was sonicated with phosphatidylcholine (0.65 mM) and BSA (0.4 mg/ml). The reaction was started by the addition of substrate, including 1 mM-dithiothreitol in accordance with Lawson *et al.* [34]. Controls were composed and incubated exactly as the samples, except that phosphatidate was omitted from the substrate. The incubation was terminated after 60 min by addition of trichloroacetic acid, and PAP activities were determined by measuring the rate of P_i production; 1 m-unit corresponds with the production of 1 nmol of P_i /min. The phosphate hydrolysis was nearly linear with time for at least 60 min, and the reaction velocity was proportional to the amount of enzyme over the range 0–1.5 mg of protein/ml. In PAP-translocation experiments, post-mitochondrial supernatant fractions were incubated with different amounts of oleic acid or KCl during 60 min at 37°C . After the incubation, microsomes and supernatant were separated by centrifugation and PAP activities were measured in the microsomal pellet and the supernatant.

DGAT activity was measured by a modification of the method of Coleman & Bell [35]. Assays were performed in stoppered reaction vials at 30°C . Each vial contained 75 mM-Tris/HCl, pH 7.4, 6 mM-MgCl₂, 0.10 mM-[³H]palmitoleoyl-CoA and BSA (5 mg/ml). The reaction was started by addition of 20 μl of 10 mM-dipalmitoleoylglycerol in ethanol, or 20 μl of ethanol (control), and 50 μl of sample protein. The reaction was terminated after 15 min by addition of 750 μl of methanol/chloroform (2:1, v/v, including 0.75 mM-tripalmitoleoylglycerol). After extraction of the lipids with chloroform/methanol, triacylglycerol was isolated by t.l.c. on silica-gel G with heptane/diethyl ether/acetic acid (60:40:1, by vol.). Triacylglycerol was scraped from the plate and counted for radioactivity in a liquid-scintillation counter. One m-unit of DGAT activity corresponds to the formation of 1 nmol of triacylglycerol/min. The assay was linear with time for 15 min, and the reaction velocity was

proportional to the amount of protein. Protein was measured by the method of Lowry *et al.* [36], with BSA as standard, and P_i was measured as described by Call & Williams [37].

Statistics

Some of the results are given as means $\pm$ s.d. Student's *t* test was used for determination of significance: $P > 0.05$ was considered not significant.

RESULTS

To study the subcellular distribution of PAP and DGAT, isolated rat hearts were perfused for 15 min, homogenized, and subcellular fractions were prepared by differential centrifugation. As presented in Fig. 1(a), about 60% of the PAP activity was recovered in the cytosolic and 20% in the microsomal fraction (Fig. 1a). The highest relative specific activity (RSA) was found in the microsomal fraction. Only 7% of DGAT activity was recovered in the cytosolic fraction, and the highest RSA was present in the microsomal fraction (Fig. 1b). Table 1 presents the kinetic properties (K_m and V_{max}) of PAP and DGAT activities. The apparent K_m of PAP for phosphatidic acid was 0.14 mM for both the cytosolic and the microsomal enzyme; the apparent K_m of DGAT for palmitoleoyl-CoA was 3.8 μM , two orders of magnitude lower than the K_m of PAP.

Fig. 1. Subcellular distribution of myocardial PAP (a) and DGAT (b) activities

Subcellular fractions were prepared from homogenates of isolated rat hearts. Relative specific activity (RSA) is defined as percentage of enzyme activity/percentage of protein. Fractions: N, nuclear; M, mitochondrial; L, lysosomal; P, microsomal; S, cytosolic. Results are means $\pm$ s.d. for four different experiments.

Table 1. Kinetic properties of PAP and DGAT activities in rat heart

PAP in myocardial microsomes and in post-microsomal supernatant (cytosol) and DGAT in post-nuclear supernatant (PNS) were incubated with various substrate concentrations. From the Lineweaver-Burk plots the kinetic parameters were calculated. Values are means of two experiments performed in duplicate.

Enzyme	K_m (mM)	V_{max} (nmol/min per mg of protein)
PAP (microsomes)	0.14	0.167
PAP (cytosol)	0.14	0.083
DGAT (PNS)	0.0038	278.2

Fig. 2. Effect of oleic acid on the activity of microsomal (●) and soluble (○) PAP activity in the myocardium

Results are means $\pm$ s.d. for four different experiments: * $P < 0.05$.

Fig. 3. Effect of oleic acid on the activity of DGAT in rat heart

DGAT activity was measured in the post-nuclear supernatant. The results were confirmed in three further experiments.

The effects of fatty acids on both enzyme activities, PAP and DGAT, are presented in Figs. 2 and 3. The PAP activity in the microsomes was increased by added oleic acid, reaching a maximum at about 0.75 mM. Increasing concentrations of oleic acid inhibited enzyme activity. The PAP activity in the cytosol, on the other hand, declined with increasing amounts of oleic acid. Myocardial DGAT activity was stimulated by oleic acid,

Fig. 4. Effect of Mg^{2+} on PAP activity in the myocardium

PAP activity was measured in the microsomal (●) and cytosolic (○) fractions with different amounts of Mg^{2+} . The Figure represents a typical example of four different experiments.

Fig. 5. Effects of (a) KCl and (b) oleic acid on the distribution of myocardial PAP activity between the soluble and the microsomal fraction

A post-mitochondrial supernatant fraction was incubated during 15 min with increasing amounts of KCl (a) or oleic acid (b). After the incubation, the microsomes were separated from the cytosol by centrifugation and PAP activity was measured in both fractions. Activity: ●, total; ■, microsomal; ▼, cytosolic. Results are means $\pm$ s.d. for four different experiments.

with an optimum at about 1 mM-oleate, and declined only slightly with increasing amounts.

Optimal PAP activity in liver and adipose tissue was dependent on the Mg^{2+} concentration during incubation. PAP activity in the myocardial microsomal fraction was optimal at 1.0 mM- Mg^{2+} (Fig. 4). Also the cytosolic enzyme was stimulated by Mg^{2+} , with an optimal Mg^{2+} concentration of 1.0 mM.

In liver and adipose tissue PAP activity can be translocated between the microsomal and the cytosolic fraction. Fatty acids as well as KCl were demonstrated to stimulate the translocation from the cytosol to the microsomes. To study PAP translocation in rat heart, we incubated a post-mitochondrial supernatant

fraction with increasing amounts of fatty acid or KCl during 15 min. After the incubation the microsomes were separated from the cytosol by centrifugation, and PAP activities were measured in both fractions. As presented in Fig. 5, myocardial PAP activity was increased in the microsomal fraction and decreased in the cytosolic fraction, after incubation with increasing fatty acid (Fig. 5b) or KCl concentrations (Fig. 5a). Incubation with higher concentrations of oleic acid (> 0.75 mM) or palmitoleoyl-CoA (0.1–2 mM) in the translocation experiments resulted in an inhibition of PAP activity. PAP activities in the cytosol and the microsomes were inhibited by 90% and 50% by palmitoleoyl-CoA (0.5 mM) respectively (results not shown).

Translocation was also studied in isolated rat hearts. After perfusion, the hearts were homogenized in 0.163 M-sucrose/54 mM-KCl/1 mM-EDTA/1 mM-Tris/HCl, pH 7.4, instead of control buffer (0.25 M-sucrose/1 mM-EDTA/1 mM-Tris/HCl, pH 7.4), and the distribution of PAP was measured in the mitochondrial, lysosomal, microsomal and cytosolic fractions. Iso-osmotic replacement of sucrose by KCl resulted in an increased recovery of PAP in the particulate fractions (Fig. 6d). Like the above-described homogenization of the hearts in KCl-containing sucrose, perfusion of the hearts with glucagon (0.1 μ M) for 30 min and subsequent homogenization in control buffer also resulted in an enhanced recovery of PAP activity in the particulate fractions (Fig. 6c). Total PAP activity was not affected by glucagon perfusion. Myocardial DGAT activity, on the other hand, was lowered from 0.37 ± 0.03 to 0.23 ± 0.02 m-unit/mg of microsomal protein ($n = 4$, $P < 0.05$).

The effects of streptozotocin-diabetes and low-flow ischaemia on myocardial PAP and DGAT activities are presented in Table 2. The PAP activity in the post-nuclear supernatant was almost twice as high in the diabetic rats. Ischaemia did not affect PAP activity significantly. In addition to PAP, DGAT activity was

Table 2. Effect of diabetes and low-flow ischaemia on PAP and DGAT activities in rat heart

PAP and DGAT activities (in m-units/g wet wt.) were measured in post-nuclear supernatant (PNS), microsomal (P) and cytosolic (S) fractions of rat heart. Diabetes was induced 3 days before rats were killed, and ischaemia was induced *in vitro* by decreasing the myocardial flow to 1 ml/min during 30 min. After perfusion, the hearts were homogenized and enzyme activities were measured in the indicated fractions. Values are given as means $\pm$ S.D. of 4–7 experiments: * $P < 0.01$.

Treatment	PAP activity		DGAT activity
	PNS	P/S ratio	PNS
None	15.6 ± 1.1	0.28 ± 0.04	2.23 ± 0.11
Ischaemia	$20.3 \pm 1.6^*$	$0.19 \pm 0.01^*$	2.44 ± 0.26
Diabetes	$28.1 \pm 3.6^*$	$0.28 \pm 0.03^*$	$3.01 \pm 0.11^*$

Fig. 6. Effect of KCl and glucagon on the subcellular distribution of myocardial PAP activity

Subcellular fractions were prepared from isolated rat hearts, with (c) or without (a, b, d) prior perfusion with 0.1 μ M-glucagon, which were homogenized in standard buffer (a, b, c) or in 0.163 M-sucrose/54 mM-KCl/1 mM-EDTA/1 mM-Tris/HCl, pH 7.4. Relative specific activity (RSA) is defined as percentage of enzyme activity/percentage of protein. The total protein concentration and enzyme activity in the post-nuclear supernatant were taken as 100%. Fractions: M, mitochondrial; L, lysosomal; P, microsomal; S, cytosolic. Results are means $\pm$ S.D. for four different experiments.

also enhanced by diabetes. The intracellular distribution of PAP activity was not changed by diabetes, as shown by the P/S ratio, i.e. the amount of enzyme activity found in the microsomes (P) relative to that found in the cytosol (S). Low-flow ischaemia, however, significantly lowered the P/S ratio.

DISCUSSION

The metabolic pathways involved in the so-called glucose/fatty acid cycle in tissues are presented in Fig. 7 [38]. Triacylglycerols are broken down by a myocardial lipase to glycerol and fatty acids. Fatty acids are activated with CoA to acyl-CoA, which can be oxidized or re-esterified with glycerol 3-phosphate coming from glycogen and/or glucose. Glycerol 3-phosphate is incorporated into triacylglycerol in four steps. First, glycerol 3-phosphate is esterified with acyl-CoA to monoacylglycerol 3-phosphate, which is subsequently esterified to phosphatidic acid, catalysed by 1-acylglycerol-phosphate acyltransferase. Phosphatidic acid and diacylglycerol, formed by hydrolysis of phosphatidic acid, are branch-points in the synthesis of triacylglycerol, since phosphatidate as well as diacylglycerol can be converted into phospholipids. Therefore we measured and characterized activities of PAP and DGAT, the enzymes catalysing the conversion of phosphatidate into diacylglycerol and of diacylglycerol into triacylglycerol in myocardial tissue.

The overall triacylglycerol-synthesizing capacity of the heart is much lower than in liver [39], and only slightly lower than in adipose tissue, when expressed per g wet weight [35]. Although total PAP and DGAT activities in the heart are about 30% of the total activity in liver, the ratios of DGAT to PAP activity in heart, liver and adipose tissue are about the same [35]. Also, the characteristics of PAP activity in heart resemble those in liver and adipose tissue. Myocardial PAP activity is enriched in the microsomal and cytosolic fractions, and the enzyme is stimulated by Mg^{2+} . In addition, the K_m values of both soluble and microsomal PAP activity in heart are similar to the K_m of PAP in adipose tissue [33], but different from the K_m of the liver enzyme, which was much lower, 0.05 mM [40]. The K_m of DGAT in heart resembles the K_m of DGAT reported for adipose tissue, 3 μM [35].

Like the enzyme in adipose tissue and liver [29], our study indicates that in myocardial cells also translocation of the enzyme from the cytosolic to the microsomal fraction does occur. The

observed stimulation of PAP translocation by fatty acids is similar to the data obtained in liver and adipose tissue. The observed translocation in heart, however, was much smaller than the translocation found in liver and adipose tissue. In heart the increase in microsomal PAP was only 50%, whereas for liver and adipose tissue a 5-fold increase has been found [41,42]. This could not be due to a decrease in the real concentration of non-esterified fatty acid because of the presence of fatty-acid-binding protein, since higher concentrations of fatty acid were not effective (results not shown). Moreover, liver tissue also contains a large amount of fatty-acid-binding protein. Also, iso-osmotic replacement of KCl in the standard sucrose buffer resulted in a much lower translocation of PAP in the particulate fractions compared with adipose tissue [41]. At the lowest ionic strength, 60% of the PAP activity was recovered in the cytosolic fraction, whereas with KCl replacement only 45% of the total PAP activity was soluble, which differs also from the change in distribution in adipose tissue, where a 5–6-fold increase in microsomal PAP activity has been found [41]. Therefore it can be concluded that the maximal translocation in heart is much lower than the translocation in liver and adipose tissue. The translocation is proposed to play an important role in the regulation of PAP, since the triacylglycerol synthesis is believed to take place in the endoplasmic reticulum [27]. Probably the translocation of PAP activity from the cytosol to the microsomes is needed for a fast elevation of the triacylglycerol-synthesizing capacity. In the myocardium triacylglycerol synthesis is necessary when the supply of fatty acids exceeds the amount of fatty acids that can be oxidized in the mitochondria. In liver and adipose tissue, however, the triacylglycerol-synthesizing capacity must be much higher for the secretion and storage of triacylglycerols. Besides the translocation, fatty acids stimulate microsomal PAP activity, analogous to the situation in liver and adipose tissue [29]. The stimulation of myocardial DGAT by fatty acids resembles the increase in hepatic DGAT activity in isolated hepatocytes exposed to fatty acids [30].

In diabetic rats PAP activity is enhanced in liver [43,44] and lowered in adipose tissue [45]. Glucagon, raised during diabetes, increased the amount of microsomal PAP and lowered DGAT activity in liver and adipose tissue [45–48]. Also, in heart total PAP activity was increased, without a change in its distribution between soluble and microsomal fractions. Besides PAP activity, DGAT activity was increased in the diabetic-rat hearts. Glucagon, however, enhanced the amount of particulate PAP activity and lowered DGAT activity in the myocardium. Therefore the increase in myocardial PAP activity cannot be explained by the enhanced glucagon level. In liver the observed increase in PAP activity in diabetes has been proposed to be due to an increase in the synthesis of the enzyme induced by glucocorticoids, since in liver actinomycin D reversed the increase in PAP activity [48]. So the increase in myocardial PAP activity seen in diabetes may also be due to an effect of the glucocorticoids. Moreover in diabetes, *in vivo*, the supply of fatty acids is also enhanced, which may lead to an additional increase in PAP and DGAT activities. Besides PAP and DGAT the synthesis of acyl-CoA is stimulated during diabetes because of an increased synthesis of CoA, found in diabetic-rat hearts [49]. All these factors lead to enhanced triacylglycerol synthesis in the myocardium in diabetes.

Myocardial PAP and DGAT activity was not significantly influenced by low-flow ischaemia *in vitro* during 30 min. The P/S ratio of PAP, however, decreased, indicating translocation of PAP activity from the reticular system to the cytosol. The relevance of PAP translocation during ischaemia and its effect on the site of triacylglycerol synthesis remain to be investigated. *In vivo*, however, PAP and DGAT activities may be enhanced during ischaemia, owing to the increased supply of fatty acids by

Fig. 7. Enzymes involved in the glucose/fatty acid cycle

(1) Glycerol-3-phosphate acyltransferase; (2) monoacylglycerol-3-phosphate acyltransferase; (3) PAP; (4) DGAT; (5) lipase; (6) acyl-CoA synthetase.

the circulation that takes place under this condition. Moreover, during myocardial ischaemia also, the supply of glycerol 3-phosphate is enhanced, owing to the altered redox state [4]. This may subsequently lead to an enhanced rate of re-esterification, as lactate addition also leads to an enhanced re-esterification [50]. Both ischaemia and diabetes are characterized by the accumulation of triacylglycerol in the heart, probably as a consequence of an enhanced rate of fatty acid re-esterification.

Besides this enhanced re-esterification, the degradation of myocardial triacylglycerols is also increased during myocardial ischaemia [5,20,21] or diabetes [22–24] and by glucagon and glucocorticoids [51]. In the myocardium glucocorticoids have a synergistic effect on the increase of heparin-releasable lipoprotein lipase activity by catecholamines [52]. Furthermore, glucocorticoids have been shown to be a permissive factor for hormone-sensitive lipolysis [51,53], leading to an enhanced lipolysis. Both the increase in lipoprotein lipase activity and lipolysis of endogenous myocardial triacylglycerols would lead to an enhanced amount of non-esterified fatty acids in the myocardium. Therefore the observed increase in myocardial PAP and DGAT activities, leading to an enhanced re-esterification of the non-esterified fatty acids and subsequently an enhanced turnover of triacylglycerols, protects the heart against the deleterious effects of high amounts of non-esterified fatty acids.

An increased triacylglycerol turnover in isolated rat hearts has also been induced by the addition of catecholamines [25,26]. These above data suggest that regulation of myocardial endogenous triacylglycerol homeostasis occurs by a glucose/fatty acid cycle (Fig. 7). Newsholme & Crabtree [54] have demonstrated that regulation by substrate cycling amplifies the enzymic control capacity of a metabolic pathway. In addition to such a metabolic amplification, the glucose/fatty acid cycle provides the heart with a mechanism that may protect against the deleterious effects of elevated levels of intracellular non-esterified fatty acid.

In conclusion, our study for the first time describes and characterizes PAP and DGAT, enzymes that, in addition to glycerol 3-phosphate acyltransferase, are involved in myocardial triacylglycerol synthesis. The relevance of both enzymes, and in particular the distribution of PAP, to the triacylglycerol/fatty acid cycling has been indicated. The rate of myocardial glucose/fatty acid cycling is proposed to be mediated by the level of non-esterified fatty acids in the tissue.

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