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Hypoxia-induced fatty acid transporter translocation increases fatty acid transport and contributes to lipid accumulation in the heart

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Abstract Protein-mediated LCFA transport across plasma membranes is highly regulated by the fatty acid transporters FAT/CD36 and FABPpm. Physiologic stimuli (insulin stimulation, AMP kinase activation) induce the translocation of one or both transporters to the plasma membrane and increase the rate of LCFA transport. In the hypoxic/ischemic heart, intramyocardial lipid accumulation has been attributed to a reduced rate of fatty acid oxidation. However, since acute hypoxia (15 min) activates AMPK, we examined whether an increased accumulation of intramyocardial lipid during hypoxia was also attributable to an increased rate of LCFA uptake as a result AMPK-induced translocation of FAT/CD36 and FABPpm. In cardiac myocytes, hypoxia (15 min) induced the redistribution of FAT/CD36 from an intracellular pool (LDM) (−25%, $P < 0.05$) to the plasma membranes (PM) (+54%, $P < 0.05$). Hypoxia also induced an increase in FABPpm at the PM (+56%, $P < 0.05$) and a concomitant FABPpm reduction in the LDM (−24%, $P < 0.05$). Similarly, in intact, Langendorff perfused hearts, hypoxia induced the translocation of a both FAT/CD36 and FABPpm to the PM (+66% and +61%, respectively, $P < 0.05$), with a concomitant decline in FAT/CD36 and FABPpm in the LDM (−24% and −23%, respectively, $P < 0.05$). Importantly, the increased plasmalemmal content of these transporters was associated with increases in the initial rates of palmitate uptake into cardiac myocytes (+40%, $P < 0.05$). Acute hypoxia also redirected palmitate into intracellular lipid pools, mainly to PL and TG (+48% and +28%, respectively, $P < 0.05$), while fatty acid oxidation was reduced (−35%, $P < 0.05$). Thus, our data indicate that the increased intracellular lipid accumulation in hypoxic hearts is attributable to both: (a) a reduced rate of fatty acid oxidation and (b) an increased rate of fatty acid transport into the heart, the latter being attributable to a hypoxia-induced translocation of fatty acid transporters.

Keywords: Cardiac myocytes; FAT/CD36; FABPpm; Hypoxia; Lipids

1. Introduction

In the well-oxygenated heart, long chain fatty acids (LCFAs) are the preferred source of energy, accounting for 80% of its oxidation [1]. Because there is limited capacity for lipid storage in cardiac myocytes, the uptake and oxidation of fatty acids are tightly coupled. An accumulation of excess lipid within cardiac myocytes can occur when the rate of fatty acid uptake exceeds the rate of fatty acid oxidation, as has been observed in heart of obese Zucker rats [2]. Recently it was shown that lipid accumulation might be a significant feature of heart failure [3]. The reason seems to be that intramyocardial lipid overload significantly contributes to contractile dysfunction [4] and arrhythmias [5].

LCFAs are known to be taken up by cells through passive diffusion [6,7]. However recent evidence has also shown that LCFA uptake is also facilitated by a protein-mediated mechanism [8–10]. Specifically, the plasmalemmal abundance of LCFA transporters determines the cellular capacity for fatty acid uptake in tissues that are highly dependent on lipid utilization [10–12]. Key LCFA transport proteins in heart include fatty acid translocase, the homolog of CD36 (FAT/CD36) and plasma membrane associated fatty acid binding protein FABPpm. Recent studies have shown that when the needs for energy are challenged in cardiac myocytes the rates of LCFA transport are upregulated, due to the translocation of FAT/CD36 from its intracellular depot to the plasma membrane [13–15]. However, it has now also been shown that a second fatty acid transporter, namely FABPpm, can be translocated from its intracellular pool(s) to the sarcolemma [16]. Interestingly, activation of specific signalling pathways selectively induces the translocation of FAT/CD36 and FABPpm. In cardiac myocytes insulin stimulation (activation of PI3K pathway) induced only FAT/CD36 to translocate to the sarcolemma, whereas stimulation with AICAR (activation of AMPK pathway) induced both FAT/CD36 and FABPpm to be translocated to the plasma membrane [16].

It is well known that during myocardial ischemia fatty acids are diverted from β-oxidation into the myocardial lipids depots [17–19]. Nevertheless, the exact mechanism leading to lipid deposition in ischemic cardiac tissue may however be more complex than simply a reduced ability to oxidize fatty acids. Under normal oxygen-supply conditions, AMPK activation results in an increased rate of fatty acid oxidation [20–22], which is supported by a concomitantly increased rate of fatty

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acid transport into the cardiac myocyte [15,16]. This latter process is facilitated by FAT/CD36 and FABPpm, which are induced to translocate to the plasma membrane, when AMPK is activated pharmacologically [15,16]. AMPK is also activated when oxygen availability is reduced in the heart or skeletal muscle, because of the increase in AMP/ATP ratio [23,24]. Under these circumstances intramyocardial lipids could accumulate rapidly, not only because of: (a) a reduced rate of fatty acid oxidation, as has previously been suggested [17,18] but (b) also due to an increased rate of fatty acid transport, as a result of the AMPK-induced translocation of FAT/CD36 and FABPpm to the cell surface. Therefore, we investigated the effects of acute hypoxia (15 min) on the rates of fatty acid transport and metabolism, and on the subcellular distribution of the fatty acid transporters FAT/CD36 and FABPpm.

2. Materials and methods

2.1. Materials

FAT/CD36 and FABPpm were detected using the MO25 antibody [25] and FABPpm antisera [26], respectively. [1-^{14}C]-palmitate and [^{3}H]-palmitate were purchased from Amersham Life Science (Little Chalfont, UK). BSA (fraction V, essentially FA free) and phloretin were obtained from Sigma–Aldrich (St. Louis, MO). Collagenase type II was purchased from Worthington (Lakewood, NJ, USA). Phosphorylated quantities of selected proteins were determined with commercially available antibodies phospho-Akt1/2/3 (Ser473), Santa Cruz Biotechnology, (Santa Cruz, CA), phospho-ERK1/2 (Thr202/Thr204) and phospho-AMPK (Thr172), Cell Signaling Technology (Beverly, MA). Appropriate secondary antibodies were purchased from Santa Cruz Biotechnology. All other chemicals were obtained from Sigma–Aldrich. Male Wistar rats (250–300 g) were bred on site and maintained at 20 °C on a reverse light–dark cycle in approved animal holding facilities. They had unrestricted access to food and water. This study was approved by the committee on animal care at the University of Guelph and at the Medical University of Bialystok, respectively.

2.2. Methods

2.2.1. Initial rates of palmitate uptake and palmitate metabolism

Initial rate of palmitate uptake by cardiac myocytes. To examine initial rates of palmitate uptake it is necessary to use cardiac myocytes [13–16]. Preparation of cardiac myocytes was performed as we have previously described [13–16]. Briefly, rats were anaesthetized using Somnotol (50–60 mg/100 g, i.p.) combined with heparin (300 i.u./100 g, i.p.). The hearts were quickly removed and placed in ice-cold Krebs–Henseleit bicarbonate buffer, (KHB, pH 7.4), and equilibrated with 95% O_2 and 5% CO_2. Subsequently, hearts were perfused (20 min) in a recirculating mode, with KHB buffer supplemented with 0.7% (w/v) BSA, 15 mM butanedione monoxime and 0.075% (w/v) collagenase type II. $CaCl_2$ was added to a final concentration 0.2 mM during the perfusion. After 20 min hearts were removed and gently minced. The suspension was incubated for another 10 min at 37 °C, while $CaCl_2$ concentration was gradually raised to 1.0 mM. Then cells were filtered through 0.2 mm nylon gauze and centrifuged for 2 min at $20 \times g$. After isolation, cells were washed twice and suspended in 20 ml of medium A (KHB buffer supplemented with 2% (w/v) BSA at a final palmitate concentration 100 μM, (palmitate/BSA ratio of 0.3) and 1 mM $CaCl_2$). At the beginning of the experiments the percentage of rod-shaped cells excluding trypan blue was determined. For all the experiments $\geqslant 80\%$ of the cardiac myocytes were structurally intact. For determination of cardiac myocytes wet mass, duplicate aliquots of the cell suspensions were centrifuged (2–3 s, at $10000 \times g$). The yield of cardiac myocytes ranged from 500 to 600 mg per single heart.

After isolation, cardiac myocytes were incubated in medium A for 15 min, continually gassed with 95% O_2 and 5% CO_2 or with 95% N_2 and 5% CO_2, and during the last 3 min of the incubation a [1-^{14}C]-palmitate (100 μmol/l with a corresponding palmitate-to-BSA ratio of 0.3)-BSA complex was added. Palmitate uptake was stopped by adding an ice-cold stop solution (KHB buffer supplemented with 0.1% BSA (w/v), 1 mM $CaCl_2$ and 0.2 mM phloretin). Subsequently, cells were washed twice with the stop solution at $60 \times g$ for 2 min. The final pellet was assayed for radioactivity.

Palmitate oxidation and incorporation into different lipid pools. To examine the effects of hypoxia on palmitate metabolism, we used intact hearts that were perfused in the Langendorff perfusion mode. After removing hearts from anaesthetized (Somnotol (50–60 mg/100 g, i.p.) combined with heparin (300 i.u./100 g, i.p.)), hearts were perfused for 5 min equilibration period with KHB, hearts were switched to medium A containing ^{3}H-palmitate (100 μmol/l with a corresponding palmitate-to-BSA ratio of 0.3) and continuously gassed for 15 min under either normoxic (95% O_2 and 5% CO_2) or hypoxic (95% N_2 and 5% CO_2) conditions. Thereafter, hearts were removed and immediately freeze-clamped in liquid nitrogen and stored at −80 °C until analyzed. Heart lipids were extracted according to van der Vusse et al. [27], with modification as we previously described [28]. Briefly, frozen heart samples were pulverized in an aluminium mortar with a stainless steel pestle, both pre-cooled in liquid nitrogen. The powder was transferred into glass tubes containing 2 ml of methanol at −20 °C. Subsequently, 4 ml of chloroform was added. Lipids from chloroform layer were separated into different fractions by means of thin-layer chromatography (silica plate 60, 0.25 mm, Merck) using heptane:isopropyl ether:acetic acid (60:40:3, v/v/v) (for TG, DAG, MG and PL separation). The total lipid extract was also separated by TLC with hexane:diethyl ether:acetic acid (10:90:1 vol/vol/vol) (for ceramide). After drying, the plates were sprayed with a 0.2% solution of 2′7′-dichlorofluorescein in methanol and exposed shortly to ammonia vapours. The lipid bands, localized under UV light and identified according to the standards (Sigma), were scraped off the plates. Finally, samples were dissolved in hexane and the ^{3}H-palmitate incorporated into different lipid pools was determined.

Exogenous oxidation rates of ^{3}H-palmitate were determined by measuring the release of $^{3}H_2O$ into the perfusate buffer. The validity of using rates of $^{3}H_2O$ production from ^{3}H-palmitate as a measure of exogenous palmitate oxidation has been previously described [29]. In brief, $^{3}H_2O$ was separated from ^{3}H-palmitate by treating 0.5 ml buffer samples with a mixture of chloroform and 2 M KCl:HCl solution. The aqueous phase was then collected and subsequently treated with a mixture of chloroform, methanol and KCl:HCl (1:1:0.9 vol/vol/vol). Subsequently, the aqueous phase was removed and counted for radioactivity. This technique results in an extraction and separation of $^{3}H_2O$ from ^{3}H-palmitate, as has been reported previously [29].

Glycogen utilization. To examine the effects of hypoxia on glycogen utilization, we used intact hearts that were perfused in the Langendorff perfusion mode. Hearts were perfused for 5 min equilibration period with KHB and afterwards hearts were switched to medium A containing non-labeled palmitate conjugated with BSA (final palmitate concentration 100 μM, palmitate/BSA ratio of 0.3) and continuously gassed for 15 min under either normoxic (95% O_2 and 5% CO_2) or hypoxic (95% N_2 and 5% CO_2) conditions. Thereafter, hearts were removed and tissue glycogen was determined accordingly [30]. Briefly, hearts were digested for 20 min in hot 30% KOH and subsequently, a spectrophotometric assay was used to determine the content of free glucose. Glycogen level is expressed in μmol of glucose/g of tissue.

2.2.2. Subcellular fractionation of intact perfused hearts and cardiac myocytes.

To relate changes in the initial rates in palmitate uptake in cardiac myocytes and in palmitate metabolism in perfused hearts, we examined the subcellular distribution of fatty acid transporters in cardiac myocytes and in perfused hearts. For these purposes control and hypoxic hearts and cardiac myocytes were prepared as described above, frozen in liquid nitrogen and stored at −80 °C until analyzed.

Subcellular fractionation of cardiac myocytes. Subcellular fractionation of control and hypoxic cardiac myocytes was performed as we have described previously [13–16,31]. Briefly, at the end of incubation NaN_3 was added (final concentration 5 mM). Collected cardiac myocytes were frozen in liquid N_2, and kept at −80 °C until analyzed. To detect the subcellular distribution of fatty acid transporters, plasma membranes (PM) and low density microsomes (LDM) were isolated from cardiac myocytes [13–16,31]. We have previously examined the purity of the fractionation procedure in which we characterized PM and LDM fractions using immunoblotting and enzymatic measurements [13–16].

Subcellular fractionation of intact perfused hearts. Subcellular fractionation of control and hypoxic Langendorff perfused hearts was performed using procedures previously described [32]. Upon thawing, hearts were diced and incubated for 30 min in a high-salt solution (2 mol/l NaCl, 20 mmol/l HEPES pH 7.4, and 5 mmol/l NaN_3) at 4 °C as recommended by Fuller et al. [32]. Thereafter, the suspension was centrifuged for 5 min at $1000 \times g$ and the pellet homogenized in 6.0 ml TES-buffer using a tightly fitting 10-ml Potter-Elvejhem glass homogenizer with 10 strokes. The resulting homogenate was centrifuged for 5 min at $1000 \times g$, after which the pellet was rehomogenized in 4.0 ml TES-buffer with 10 strokes and then recombined with the $1000 \times g$ supernatant. The homogenate was centrifuged for 10 min at $100 \times g$. The pellet (P1) was resuspended in 300 μl TES-buffer and saved. The supernatant was centrifuged for 10 min at $5000 \times g$. The pellet (P2) was resuspended in 300 μl TES buffer and saved. The supernatant was centrifuged for 20 min at $20\,000 \times g$. The pellet (P3) was resuspended in 300 μl TES-buffer and saved. The supernatant was centrifuged for 30 min at $48\,000 \times g$. The pellet (P4) was resuspended in 150 μl TES-buffer and saved. The supernatant was centrifuged for 65 min at $250\,000 \times g$. The pellet (P5) was resuspended in 150 μl TES-buffer and saved. Upon analysis of P1–P5 with ouabain-sensitive *p*-nitrophenyl-phosphatase and with EGTA-sensitive Ca^{2+}-ATPase,it has been established that fractions P2 refers as PM-fraction and P5 as LDM fraction [13–16,33].

2.2.3. Western blotting. FAT/CD36 and FABPpm protein expressions were determined in cardiac myocytes and perfused intact hearts. For these purposes cardiac myocytes were washed twice with PBS buffer, homogenized and resuspended in Buffer 2 (Tris-base 10 mM, EDTA 1 mM, pH 7.4) and frozen in liquid nitrogen. From perfused hearts homogenates yielding crude membranes were prepared as described previously [13,33]. FAT/CD36 and FABPpm were also determined in PM and LDM fractions from subfractionated cardiac myocytes, as well as in PM and LDM obtained from perfused hearts. For other proteins, cardiac myocytes at the end of the incubation period were washed twice with ice-cold PBS buffer. Proteins were extracted with extraction buffer (in mM: 50 Tris-acid base (pH 7.5), 1 EDTA, 1 EGTA, 1 sodium ortovanadate, 50 NaF, 5 sodium pyrophosphate, 270 sucrose, 1 DTT, 1% Triton X-100, 5 μg ml leupeptin, 5 μg/ml pepstatin) as described [13,33]. The supernatant fraction was collected after centrifugation at $10\,000 \times g$, for 10 min. at 4 °C and frozen in liquid nitrogen for protein assay and Western blot protocols. Routine Western blotting procedures were used to detect proteins as described previously [13–16]. Protein content was determined with bicinchonic acid method with BSA serving as a protein standard Signals obtained by Western blotting were quantified by densitometry (SynGene, ChemiGenius2, Perkin–Elmer, Boston, ON).

All data are expressed as means ± S.E.M. Statistical difference between groups was tested with analyses of variance and appropriate post hoc tests, or with a *t*-test. Statistical significance was set at $P \leqslant 0.05$.

3. Results

3.1. Effects of hypoxia on the rates of LCFA uptake and metabolism

During hypoxia, (15 min) the initial rate of palmitate uptake by cardiac myocytes was increased (+40%, $P < 0.05$, Fig. 1A). In contrast, the rate of palmitate oxidation during acute hypoxia was reduced (−35%, $P < 0.05$, Fig. 1B). Acute hypoxia induced also a reduction in myocardial glycogen (−24%, $P < 0.05$, Fig. 1C).

Hypoxia (15 min) increased palmitate incorporation into phospholipids (+48%, $P < 0.05$, Fig. 2A) and into the intracellular triacylglycerol depot (+28%, $P < 0.05$, Fig. 2A). Acute hypoxia induced a parallel increase in palmitate incorporation into ceramides (+21%, $P < 0.05$, Fig. 2B). No significant changes in other lipid fractions were observed. Total lipid accumulation, calculated as the sum of all neutral lipid fractions, was also increased (+26%, $P < 0.05$, Fig. 2C).

3.2. Effects of hypoxia on the subcellular distribution of fatty acid transporters (FAT/CD36, FABPpm)

Hypoxia (15 min) did not alter the total protein expression of FAT/CD36 and FABPpm, either in isolated cardiac myocytes or in intact, perfused hearts (data not shown). However, incubation of cardiac myocytes under hypoxic conditions induced the translocation of FAT/CD36 from the LDM (−25%, $P < 0.05$) to the PM (+54%, $P < 0.05$) in cardiac myocytes (Fig. 3A). We also observed a hypoxia-induced increase in FABPpm at the PM (+56%, $P < 0.05$) and a concomitant FABPpm reduction in the LDM (−24%, $P < 0.05$) in cardiac myocytes (Fig. 3B). Importantly, in intact Langendorff perfused hearts, a more physiological preparation, hypoxia also

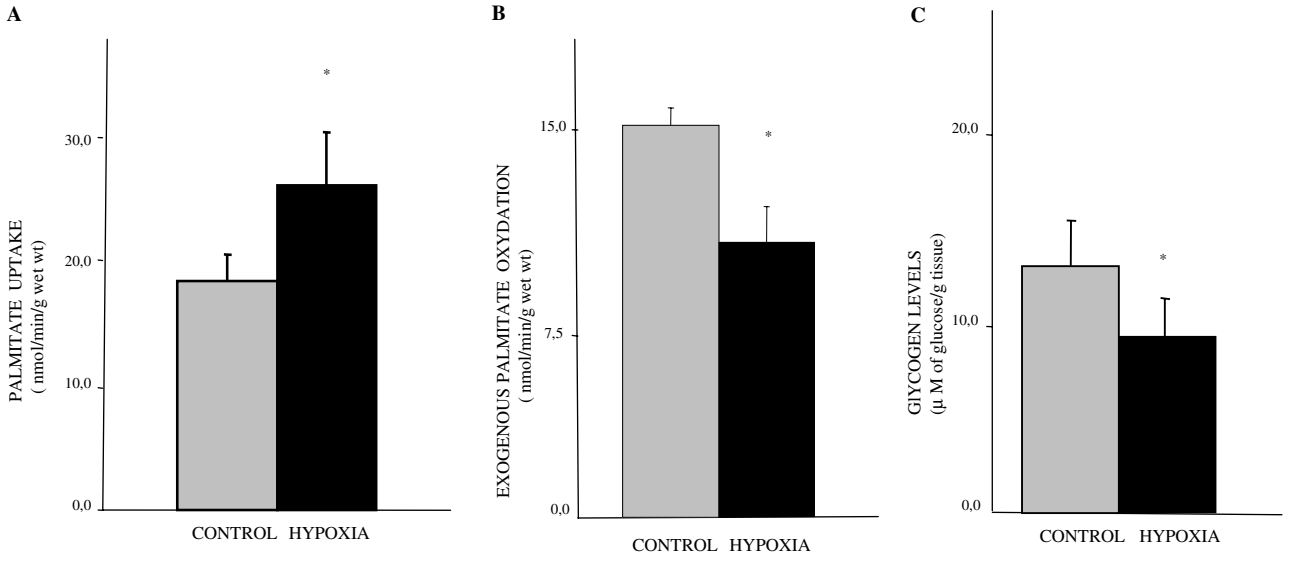

Fig. 1. Effects of hypoxia (15 min) on (A) the initial rates of palmitate uptake by cardiac myocytes, (B) exogenous palmitate oxidation and (C) myocardial glycogen utilization in Langendorff perfused hearts. Data are based on five independent determinations for each treatment (means ± S.E.M.). *$P < 0.05$, hypoxia vs. control.

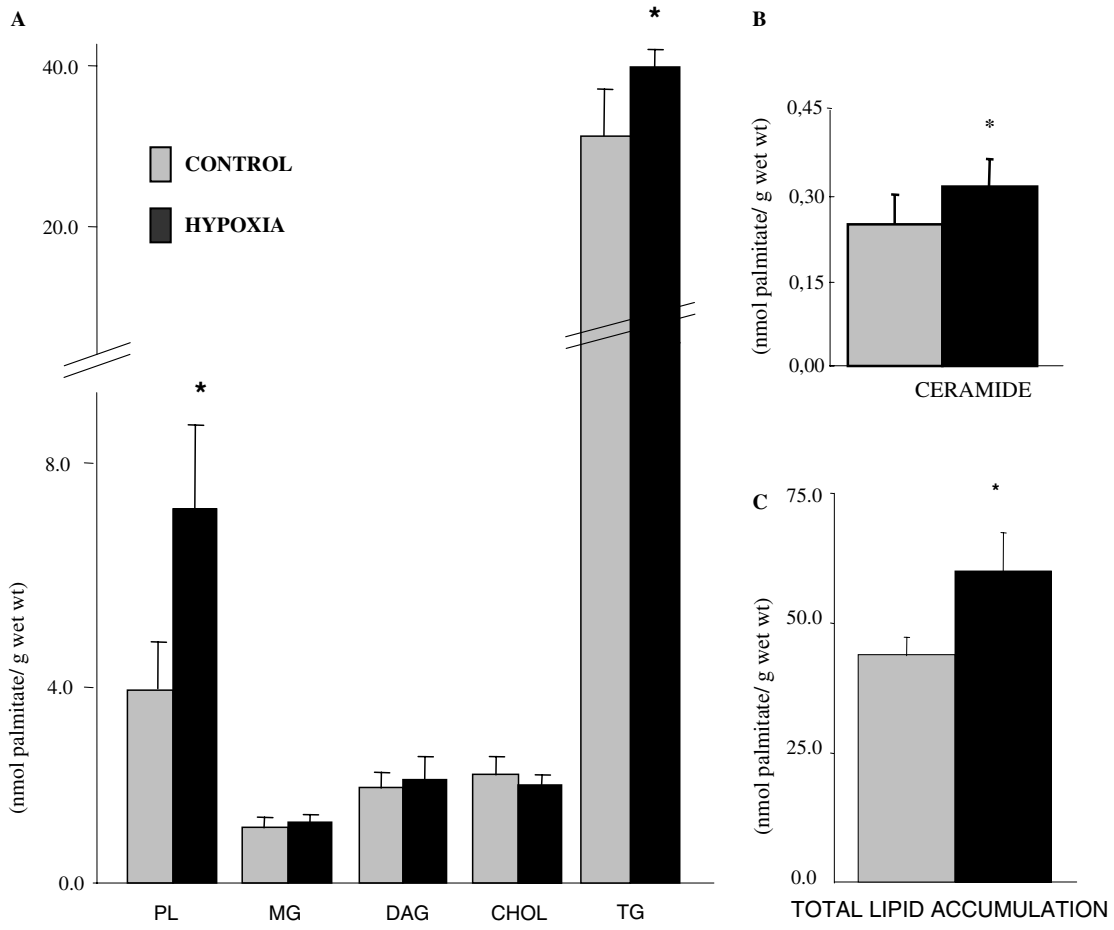

Fig. 2. Effects of hypoxia (15 min) on palmitate incorporation into different intracellular lipid pools in Langendorff perfused hearts. Data are based on five independent determinations for each treatment (means ± S.E.M.). $^{*}P < 0.05$, hypoxia vs. control.

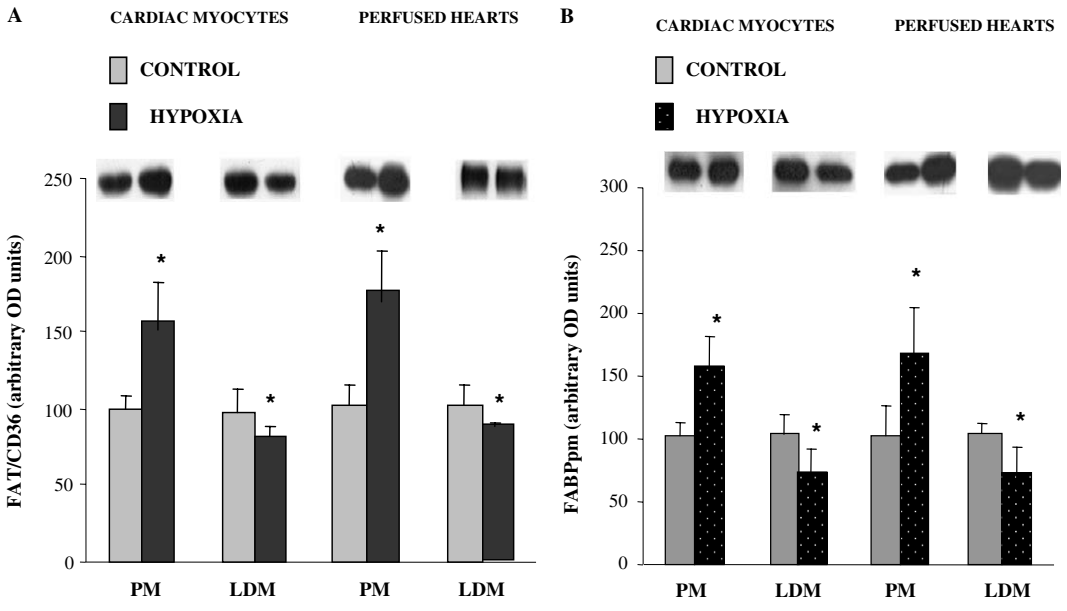

Fig. 3. Subcellular redistribution of FAT/CD36 and FABPpm in hypoxic (15 min) (A) cardiac myocytes and hypoxic (15 min) (B) Langendorff perfused hearts. Representative Western blots are also shown. Data are based on five independent determinations (means ± S.E.M.). PM, plasma membranes; LDM, low density microsomes. $^{*}P < 0.05$, hypoxia vs. control.

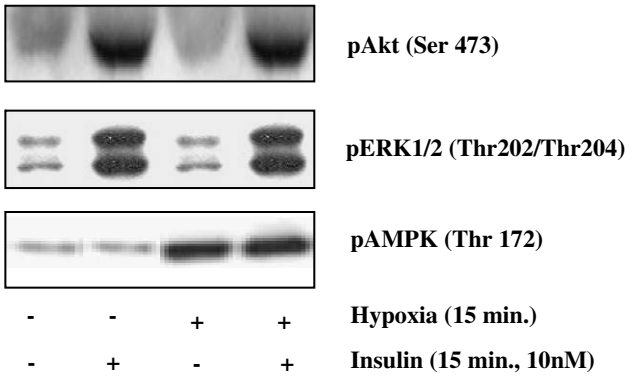

Fig. 4. Representative Western blots showing the effects of hypoxia on the phosphorylation of AMPK (Thr172), ERK1/2 (Thr202/Thr204) and pAkt (Ser473) proteins in cardiac myocytes. The same quantity of protein (30 μg) is loaded into each well and check for equal loading after transfer with Ponceau stain. Similar results were observed in three independent experiments. Insulin effects are included as a positive control.

induced the translocation of a both FAT/CD36 and FABPpm to the PM (+66% and +61%, respectively, $P < 0.05$, Fig. 3A and B), with a concomitant decline in FAT/CD36 and FABPpm in the LDM (−24% and −23%, $P < 0.05$, Fig. 3A and B).

3.3. Effects of hypoxia on signaling pathways in cardiac myocytes

We examined possible signaling pathways activated by acute hypoxia (15 min) in cardiac myocytes. As has been shown previously [24,33], exposure of cardiac myocytes to hypoxia increased the AMPK phosphorylation (Fig. 4). Hypoxia did not induce the phosphorylation of ERK1/2 nor Akt (Fig. 4). As a positive control, acute exposure of cardiac myocytes to insulin (10 nM, 15 min) did stimulate the phosphorylation of Akt ERK1/2, as expected [35–37].

4. Discussion

In the present study we confirmed that hypoxia reduced palmitate oxidation and this was associated with an increase in palmitate incorporation into selected lipid depots (phospholipids, triacylglycerols and ceramides). In addition, we have the novel findings that: (a) during hypoxia the initial rate of palmitate uptake is upregulated, and that (b) this is associated with a concomitant hypoxia-induced translocation of the fatty acid transporters FAT/CD36 and FABPpm from low density microsomes, an intracellular depot, to the plasma membrane. Given that hypoxia activated AMPK [34], and based on our previous work [15,16,33], we propose that the hypoxia-induced activation of AMPK is the molecular mechanism that stimulates the translocation of the fatty acid transporters from their intracellular depots to the cell surface. Thus, the present studies have shown that an increased rate of LCFA transport, as well the previously known reduction in LCFA oxidation, both contribute to the intramyocardial accumulation of lipids in the hypoxic heart.

It is well known that myocardial lipid accumulation during hypoxia could be at least partially due to the switch in energy substrate preference from predominantly the oxidation of fatty acids to the increased utilization of glucose [38–40]. Acute hypoxia accelerates also the glycogen breakdown as an alternative energy source [41]. Previously, it has been shown that in the ischemic myocardium fatty acids are diverted from β-oxidation into deposition as tissue lipids [17,18,42]. Our study concurs with these observations, as we observed both a decreased rate of palmitate oxidation, increased glycogen utilization and a concomitant increase in the deposition of fatty acids into myocardial phospholipid and triacylglycerol depots during acute hypoxia.

In the present study accumulation of fatty acids in the PL and TG depots may be surprising. This may be expected to reduce intracellular ATP levels, since esterification of fatty acids is energetically expensive, and ATP is already reduced during hypoxia [43]. However, removing fatty acids from cytosol could also be beneficial, since high concentrations of myocardial fatty acids may be detrimental to the cardiac myocytes [44–47]. Nonetheless, elevated myocardial triglycerides and ceramides in obese Zucker diabetic rats have been associated with the development of cardiac dilatation and reduced contractility [48]. Moreover, accumulation of intracellular ceramide concentrations alone have been also associated with myocardial damage [49], and ceramide accumulation is known to occur in hearts during ischemia/reperfusion [50,51]. Thus, it seems that removing fatty acids from the cytosol into triacylglycerol depots and ceramides during hypoxia cannot be seen as being beneficial. It is likely that the greater incorporation of fatty acids into lipid depots reflects a mass action effect due to increased uptake of LCFA into the cell and the diminished rate of LCFA oxidation during hypoxia.

Recent studies in cardiac myocytes have demonstrated that protein-mediated LCFA transport across plasma membranes is a highly regulated process involving changes in the sarcolemmal content of fatty acid transporters [13–16]. Specifically, insulin induced the translocation of FAT/CD36 only, while contraction-like stimuli (AICAR and oligomycin) induced the redistribution of both FAT/CD36 and FABPpm from an intracellular pool to the plasma membrane [13–16]. These increase in plasmalemmal fatty acid transporters, whether induced by insulin or by contraction-mimetic signals, increased the rate of LCFA transport [13–16,33]. Therefore, an important aspect of the present study was to establish whether the hypoxia-induced changes in the initial rates of LCFA uptake by cardiac myocytes also resulted in redistribution of the LCFA transporters to the plasma membranes. In the present study, we have shown for the first time, in isolated cardiac myocytes as well as in the intact perfused heart, that hypoxia induced the translocation of both FAT/CD36 and FABPpm from an intracellular pool to the plasma membrane, an effect that was not attributable to the increased expression of these proteins (data not shown). It is likely that the redistribution of FAT/D36 and FABPpm to the plasma membranes in hypoxic cardiac myocytes and hypoxic hearts can be attributed to the hypoxia-induced AMPK activation, since we (present study) and others [34,46,47,52] have observed that myocardial hypoxia triggers the activation of AMP-kinase, and since we have previously shown that AMPK activation redistributes FAT/CD36 and FABPpm to the plasma membrane [16,33].

Previous studies from our laboratories have shown that rates of LCFA uptake are increased whenever the plasmalemmal content of fatty acid transporters is increased, and conversely that the rate of LCFA transport is reduced when the plasmalemmal content of fatty acid transporters is decreased

[13–16,53,54]. Thus, the hypoxia-induced increase in the initial rate of LCFA uptake is most likely attributable to the hypoxia-induced translocation of LCFA transporters to the plasma membrane.

5. Conclusions

In present study we have provided several novel observations. First, we have shown that acute hypoxia induces the redistribution of FAT/CD36 and FABPpm from an intracellular pool to the plasma membranes, an effect that was most likely triggered by the activation of AMPK in the hypoxic myocyte. Second, the translocation of the LCFA transporters to the plasma membrane increased the initial rates of LCFA uptake into cardiac myocytes in proportion to the increase in the plasmalemmal content of two fatty acid transporters (FAT/CD36 and FABPpm). A quantitatively similar, hypoxia-induced translocation of FAT/CD36 and FABPpm was observed in intact perfused heart. Finally, the rates of fatty acid oxidation were also reduced in the hypoxic heart while fatty acid incorporation into PL, TG and ceramides was increased. Thus, our data indicate that the increased intracellular lipid accumulation in hypoxic hearts is attributable to: (a) not only a reduced rate of fatty acid oxidation, as had been shown elsewhere, but also to (b) an increased rate of fatty acid transport into the heart, the latter being attributable to a hypoxia-mediated activation of AMPK which induces translocation of fatty acid transporters.

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