Original Article

Myocardial Expression of Heart-Type Fatty Acid Binding Protein (h-FABP) in Various Cardiac Stress Conditions in Rats

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Abstract

Objective: The heart-type fatty acid binding protein (h-FABP) has recently been studied as the specific biomarker of myocardial injury. The present study aimed to investigate the potential stress conditions that affect the expression of h-FABP in the rat heart.

Methods: Immunoblot analysis was used to quantify the protein expression of h-FABP in the heart under various cardiac stress conditions, including the effect of an aerobic training program, deprivation of ovarian sex hormones, angiotensin II-induced hypertension, doxorubicin-induced cardiotoxicity, and type II diabetes.

Results: No significant change in h-FABP protein expression in the heart was found after a 9-week exercise training compared to sedentary controls. Lack of female sex hormones for 10 weeks also had no effect on h-FABP protein expression. In addition, there was no change in h-FABP expression due to either 4-week angiotensin II infusion or doxorubicin treatment compared to their vehicle controls. A significant increase in h-FABP was demonstrated in the heart of spontaneous diabetes Torii (SDT) rats compared to those without diabetes (*P*-value < 0.0001). There was a high correlation between degree of hyperglycemia and the myocardial expression of h-FABP protein ($r^2 = 0.9252$, *P*-value = 0.0022).

Conclusion: The findings suggest that the expression of h-FABP in the heart is primarily regulated by available sources of energy, while cardiac hypertrophy and myocardial damage do not particularly contribute to h-FABP expression.

Keywords: Exercise training, Doxorubicin, Angiotensin II, Ovariectomy, Diabetes, Heart-type fatty acid binding protein (h-FABP)

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Introduction

In healthy myocardium, approximately 60-90% of ATP are derived from long-chain fatty acid oxidation to maintain cardiac function during rest.[1] Since cardiomyocytes have small fatty acids (FA) storage, the heart is particularly reliant upon circulating FA. Fatty acids are transported across the membrane by two major membrane proteins, including fatty acid transporter protein (FATP) and fatty acid translocase (FAT/CD36). Via FAT/CD36, FA is bound to the fatty acid binding protein (FABP) in order to transport FA from the sarcolemma to the outer mitochondria membrane and is rapidly converted into acyl-CoA. Alternatively, FA entering via FATP are immediately converted to acyl-CoA. It has been reported that the dysfunction of FATP, FAT/CD36, and FABP could limit cellular activity, especially in cardiac muscle.[2]

In cardiomyocyte, FABP plays a critical role in FA uptake and intracellular transportation in which heart-type isoform is predominant.[3] Heart-type FABP deficiency led to diminished FA uptake and utilization and consequently converted cardiomyocytes to be a glucose-dependent phenotype.[4] It is therefore possible that h-FABP could have a role in the rate-limiting step in cardiac FA utilization. The present study therefore aimed to investigate the potential conditions that can regulate the expression of h-FABP in the heart using various rat models that had undergone different cardiac stresses. Physiological stress of aerobic exercise training and deprivation of female sex hormones were evaluated. While pathological stress including chronic angiotensin II-induced hypertension, doxorubicin-induced cardiotoxicity, and diabetic cardiomyopathy were examined to provide an in-depth and thorough understanding of the factors that regulate cardio-metabolic adaptation. The information would support the development of therapeutic approaches against metabolic disorders in the pathological heart.

Materials and methods

Animal models

To investigate the factors that could affect h-FABP expression, frozen rat hearts from previous experiments in the researcher's laboratory were used.[5, 6] The examined conditions include: *1) the effect of moderate–intensity exercise training:* Eight-week female Sprague-Dawley rats were divided into sedentary and nine-week moderate–intensity exercise training groups using a motor-driven treadmill, as described in a previous study,[5] one hour each day (Supplement Table. S1). *2) The effect of female sex hormone deprivation:* Eight-week female Sprague-Dawley rats were divided into sham operated control and ovariectomized groups.[5] Atrophy of uterine indicated deprivation of female sex hormones (Supplement Table. S2). *3) The effect of chronic angiotensin II treatment:* Adult female Sprague-Dawley (14-week-old) rats were divided into vehicle control and angiotensin II infused groups.[5] The rats were subcutaneously implanted with a mini-osmotic pump (ALZET model 2004) containing a vehicle (150 mM NaCl and 0.01% acetic acid) or angiotensin II at a continuous release rate of approximately 0.7 mg per kilogram of body weight per day for four weeks. Significant increases to blood pressure indicated the effectiveness of angiotensin II infusion[5] (Supplement Table. S3). *4) The effect of doxorubicin-induced cardiotoxicity:* Female Sprague-dawley rats were divided into vehicle control and doxorubicin-treated groups.[6] Doxorubicin-treated groups were intraperitoneally administered doxorubicin to induce cardiotoxicity, while normal saline was injected in the control rats. Doxorubicin was used at an accumulative dose of 15 mg/kg body weight, separated into six injections (2.5 mg/kg per injection administered every other day) based on the human clinical dose and pharmacological scale for rat usage (Supplement Table. S4). *5) The effect of type 2 diabetes:* Forty-week old spontaneously Diabetic Torii (SDT) rats, while eight-week old male Sprague-Dawley rats with fasting blood glucose lower than 120 mg/dL were used as non-diabetic controls (Supplement Table. S5).

Ethical approval

The animal protocols were approved by the Experimental Animal Committee, Faculty of Science, Mahidol University. Approved animal protocols included protocol numbers MUSC62-022-486 and MUSC61-013-415.

Supplementary materials

Table S1 General characteristics of sedentary and exercise training rats[5]

	Sedentary (n = 8)	**Exercise training (n = 8)**
Body weight (g)	229 ± 3	317 ± 5*
Systolic blood pressure (mmHg)	123 ± 3	119 ± 4
Diastolic blood pressure (mmHg)	80 ± 2	80 ± 3
Heart weight (g)	1.45 ± 0.02	1.76 ± 0.02*
Heart/Body weight	0.47 ± 0.01	0.54 ± 0.01*
Cardiomyocyte CSA# (μm^2)	185 ± 2	224 ± 3*
Plantaris citrate synthase activity (nmole/min/mg protein)	84 ± 7	128 ± 4*

Data are presented as means ± SEM (# Cardiomyocyte CSA (cross-sectional area), n = 120 cells from 5 hearts per group). *$P < 0.05$, significantly different from Sedentary, using independent t-test.

Table S2 General characteristics of sham and ovariectomized rats[5]

	Sham-operated rat (n = 8)	**Ovariectomized rat (n = 8)**
Body weight (g)	229 ± 3	353 ± 7*
Systolic blood pressure (mmHg)	123 ± 3	121 ± 2
Diastolic blood pressure (mmHg)	80 ± 2	80 ± 3
Heart weight (g)	1.45 ± 0.02	1.52 ± 0.04
Heart/Body weight	0.47 ± 0.01	0.42 ± 0.01
Cardiomyocyte CSA# (μm^2)	185 ± 2	150 ± 2*
Uterine weight (g)	0.56 ± 0.02	0.14 ± 0.01*

Data are presented as means ± SEM (# Cardiomyocyte CSA (cross sectional area) , n = 120 cells from 5 hearts per group).*$P < 0.05$, significantly different from SHAM, using independent t-test.

Table S3 General characteristics of vehicle control and Angiotensin II infusion rats[5]

	Vehicle control (n = 8)	**Angiotensin II infusion (n = 8)**
Body weight (g)	229 ± 3	294 ± 3*
Systolic blood pressure (mmHg)	123 ± 3	165 ± 6*
Diastolic blood pressure (mmHg)	80 ± 2	105 ± 8*
Heart weight (g)	1.45 ± 0.02	1.56 ± 0.04*
Heart/Body weight	0.47 ± 0.01	0.52 ± 0.01*
Cardiomyocyte CSA# (μm^2)	185 ± 2	247 ± 3*
Uterine weight (g)	0.56 ± 0.02	0.14 ± 0.01*

Data are presented as means ± SEM (# Cardiomyocyte CSA (cross-sectional area), n = 120 cells from 5 hearts per group).*$P < 0.05$, significantly different from Vehicle control, using independent t-test.

Table S4 General characteristics of vehicle-injected rat and doxorubicin-treated rat[6]

	Vehicle control (n = 11)	Doxorubicin treatment (n = 11)
Body weight (g)	359 ± 8	335 ± 12
Heart weight (g)	1.22 ± 0.03	1.12 ± 0.05
Fractional shortening %	39.6 ± 0.7	23.4 ± 1.6*
Ejection fraction %	75.9 ± 0.7	52.4 ± 2.7*
Plasma LDH activity (mU/mL)	0.0479 ± 0.0018	0.0625 ± 0.0013*

Data are presented as means ± SE. *LDH*, lactate dehydrogenase. *$P < 0.05$, significantly different from vehicle controls, using independent t-test.

Table S5 General characteristics of non-diabetic control and SDT rats

	Non-diabetes rat (n = 8)	Diabetes SDT rat (n = 8)
Body weight (g)	287 ± 4	401 ± 5*
Heart weight (g)	0.91 ± 0.13	1.45 ± 0.06*
Fasting blood glucose (mg/dL)	102.5 ± 1.35	419 – 600#

Data are presented as means ± SE. *SDT*, Spontaneously Diabetic Torii.*$P < 0.05$, significantly different from non-diabetic control, using independent t-test. (Exclude one SDT rat which fasting blood glucose 227 mg/dL) #Note: the maximum limitation of blood glucose level detection is at 600 mg/dL.

Immunoblot analysis

Frozen left ventricular tissues from a -80ºC freezer were homogenized with an extraction sample buffer containing 0.5 M tris pH 6.8, 2.5% SDS, 10% glycerol, 1 µg/mL leupeptin, 1 µg/mL pepstatin, and 1 mM phenylmethylsulfonyl fluoride. Proteins in the homogenized samples were separated by SDS-PAGE. Membranes were incubated with the rabbit polyclonal antibody against heart-type FABP (1 : 400, Abcam; ab45966) and polyclonal rabbit antibodies directed against beta-actin (1 : 5000, Cell Signaling #4967), followed by secondary antibodies, anti-rabbit (1:10000, Cell Signaling #7074). Protein bands were developed using enhanced chemiluminescence reagents (ECL; Amersham Biosciences) and the band intensity was detected.

Data and statistical analysis

All data are presented by box plot. Independent t-test was performed to determine the differences between the experimental groups. The significance was set to *P*-value < 0.05.

Results

Exercise training: Since physiological cardiac stress from aerobic exercise caused an adaptation of myocardial energy utilization,[7] h-FABP expression in the cardiac muscle was then examined. Although aerobic exercise training significantly induced cardiac hypertrophy (Supplement Table S1), immunoblot analysis indicated no difference in the expression of h-FABP in the left ventricle of the trained rats after exercise as compared to sedentary control (Figure 1A). The results suggest that regular aerobic exercise at a moderate intensity was not a condition that affected h-FABP expression in the heart.

Angiotensin II infusion: Since a previous study demonstrated that angiotensin II downregulated the fatty acid oxidation pathway in adult rat cardiomyocytes,[8] the expression of h-FABP in the heart after prolonged angiotensin II overstimulation was then examined. While angiotensin II overstimulation demonstrated hypertension and hypertrophy of the heart (Supplement Table S3), there was no change in h-FABP expression in the rat heart after four weeks of angiotensin II infusion when

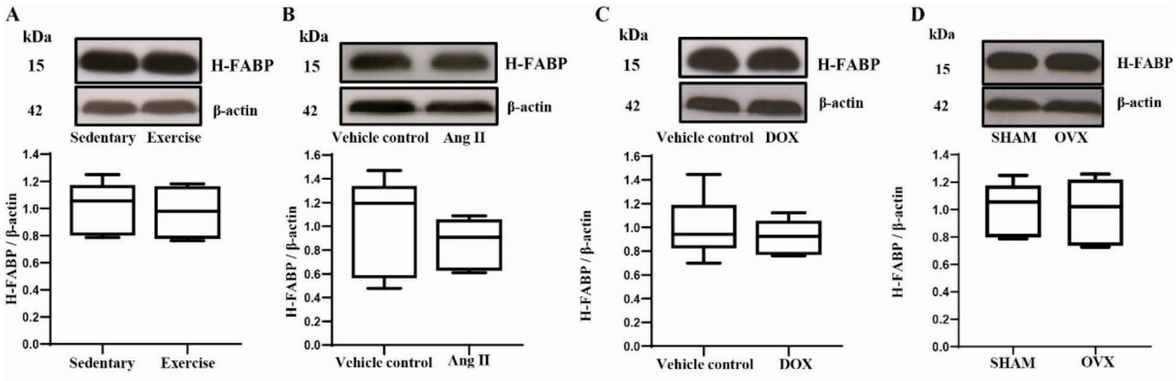

Figure 1 The effect of various cardiac stress conditions on h-FABP expression in the heart. Representative immunoblot of h-FABP in comparison with beta-actin expression: (A) Sedentary rats versus exercise training rats (n = 5). (B) Vehicle infusion versus angiotensin II infusion (Ang II) (n = 5). (C) Vehicle injection versus doxorubicin administration (DOX) (n = 6). (D) Sham-operated control versus ovariectomized rats (OVX) (n = 5). Data are summarized by box plot. No significant difference was observed using independent t-test.

compared to the vehicle-treated controls (Figure 1B). The results indicate that angiotensin II had no impact on h-FABP expression in heart.

Doxorubicin administration: Doxorubicin treatment impairs most cardiac energy metabolic processes and changes substrate utilization to glucose dependent, similar to heart failure.[9] Although myocardial function was clearly decreased (Supplement Table S4), doxorubicin administering did not change the expression of h-FABP in the heart compared to the vehicle-injected rat heart (Figure 1C). Thus, the expression of h-FABP in the heart muscle was not influenced by myocardial damage due to doxorubicin treatment.

Lack of female sex hormones: Previous studies demonstrate cardiac dysfunction with a reduction in cardiac fatty acid oxidation after deprivation of female sex hormones in ovariectomized model.[10] Therefore, the expression of h-FABP in heart was captivating. The results showed that h-FABP expression in the left ventricle of 10-week ovariectomized rats did not change when compared to sham-operated controls (Figure 1D). This result suggested that female sex hormones did not have any role in regulating myocardium h-FABP alteration.

Diabetes: Diabetes mellitus is a metabolic disorder which causes the elevation of fatty acid utilization rates.[11] Figure 2A demonstrates the expression of h-FABP from eight SDT rats with fasting blood glucose over 400 mg/dL and eight SD rats with fasting blood glucose lower than 120 mg/dL. Significant ($P < 0.0001$) increase in h-FABP expression in the heart SDT group was demonstrated when compared to the non-diabetic controls (Figure 2A). A high correlation between fasting blood glucose level and the expression of h-FABP in the heart was presented ($r^2 = 0.9252$, $P = 0.0022$) (Figure 2B). The results confirm that h-FABP expression is involved in myocardial adaptation under diabetic conditions.

Discussion

The present study aimed to determine the stress conditions that could alter h-FABP as the rate-limiting step during cardiac FA utilization. The results demonstrate that the expression of h-FABP protein in the heart was not affected by either moderate-aerobic exercise training or deprivation of female sex hormones. Overdose angiotensin II-induced hypertension had also no regulatory effect on h-FABP protein expression. Unexpectedly, the expression of h-FABP in the heart was not changed under doxorubicin-induced cardiomyopathy. Nevertheless, the results demonstrate that genetic-induced diabetic hyperglycemia significantly upregulated h-FABP expression in cardiac tissue, in which the degree of h-FABP expression was

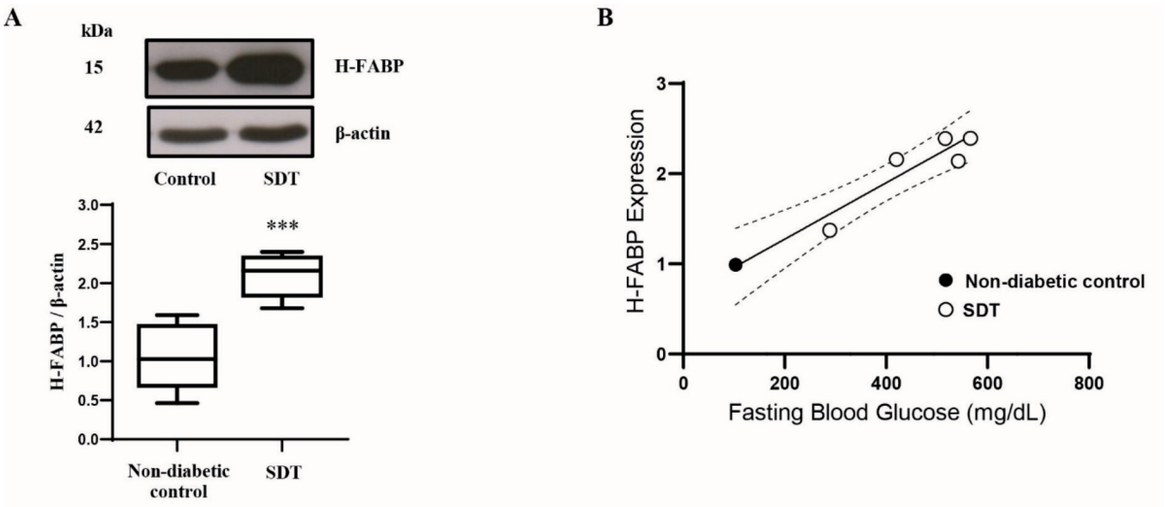

Figure 2 Effect of type-2 diabetes on myocardial h-FABP in SDT rats. (A) h-FABP expression over actin in the heart between non-diabetic control and Spontaneously Diabetic Torii rats (SDT) (****P*-value < 0.0001, $n = 8$, independent t-test). (B) Relationship between h-FABP expression in heart tissue and fasting blood glucose of non-diabetic rats (mean value) and SDT rats ($n = 5$), linear regression analysis indicated a significant relation between myocardium h-FABP expression and fasting blood glucose ($r^2 = 0.9252$), $P = 0.0022$). (Black and white dot represented mean values from non-diabetes and diabetes groups, respectively).

closely related to the glycemic level. Accordingly, the expression of h-FABP was associated with the energy source availability, but was independent from hypertrophy or cardiac dysfunction.

Physiological stress and cardiac fatty acid utilization

As an intracellular FA transporter, a change in h-FABP expression could disturb tissue energy utilization. Numerous studies have directly investigated myocardial FA utilization after exercise training. Both unaltered[12] and increased[13] FA uptake in the heart was found after exercise training. Endurance training was shown to upregulate the mRNA and the protein expression level of fatty acid transport protein (FATP),[13] which could potentially influence FA uptake in the heart. In addition, an increase in PPARα in a trained heart may contribute to the FA oxidation improvement.[14] However, the previous study reported that exercise training had no effect on h-FABP protein expression in rabbit hearts after a 4-week running program,[15] although similar interventions could improve FA utilization and upregulate h-FABP in myocardial infracted heart. Similar to the findings of the present study which involved a 9-week aerobic training program, this suggests that the volume of exercise training is not the factor that regulates h-FABP in the healthy heart.

Menopause is another physiological condition that disturbs myocardial substrate utilization. FA oxidation decline and increased accumulation of FA in human cardiomyocytes was detected after deprivation of female steroid hormones,[16] whereas hormone replacement therapy could restore FA oxidation. A previous study proposed that mitochondrial dysfunction is a critical cause for unbalanced cardiac FA metabolism in the heart of menopause specimens by reducing FA oxidation and resulting in the accumulation of fatty acyl-CoA ester.[16] However, studies into fatty acid transporter proteins in the heart after deprivation of female sex hormones remain limited. The present study was the first to reveal that female sex hormones deprivation had no effect on the expression of h-FABP in rat hearts.

Pathological stress and cardiac fatty acid utilization

In the advanced stages of heart failure, the metabolic phenotype is altered to reduced cardiac fatty oxidation with increased glycolysis and glucose oxidation.[17] Protein levels of the predominant fatty acid transporter, fatty acid translocase (FAT/

CD36), and h-FABP also decreased in addition to FA oxidation enzymes.[18] Yet it is still interesting to consider whether altered energy metabolism leads to heart failure progression or the damage caused by cardiomyocytes results in changes to transporter proteins.

Hypertension can induce a shift in the main substrate utilization in the heart from FA to glucose dependent in animals.[19] The shift toward glucose usage is likely due to a decrease of plasma lemma content and protein expression of FAT/CD36 leading to impaired heart FA uptake and utilization.[20] The researchers found no change of h-FABP expression in the hypertension model induced by angiotensin II infusion. This is similar to the previous study which found that deoxycorticosterone/salt-treated hypertensive rat models showed no change in h-FABP in the heart and kidneys.[21] Our results then indicate that either hypertension or angiotensin II overstimulation did not affect the regulation of h-FABP expression in the heart.

Similar to heart failure, doxorubicin treatment causes a decrease in fatty acid oxidation accompanied by an increase in glucose utilization in the heart in both animal models[22] and doxorubicin-treated patients.[23] Although h-FABP could be considered as a factor that affects FA utilization in doxorubicin-induced cardiotoxicity, we observed no change in the expression of h-FABP protein in the heart. However, a previous study reported that the lower dose of doxorubicin significantly decreased h-FABP mRNA expression in the rat heart.[24] This was not surprising since doxorubicin directly inhibits DNA and RNA synthesis. Unfortunately, no report on protein expression has been confirmed. Thus, the results of the present study suggest that doxorubicin did not intensely alter the h-FABP protein available in the heart.

In contrast to hypertension and doxorubicin toxicity, diabetes induces a distinct cardiac metabolic phenotype which results in elevating FA utilization in the heart. Diabetes induced myocardial triglycerides accumulation leading to impaired insulin signaling and activation of oxidative metabolism signaling pathways. Increases of mRNAs for FA oxidation proteins such as FATP1, CD36, and FACS1 was demonstrated in the heart of this model.[25] The results of the present study also found that h-FABP increased in the heart of spontaneous type II diabetes. Similar to our findings, a previous study also demonstrated the upregulation of myocardium content of h-FABP in pharmacological-induced diabetes in both insulin-dependent and insulin-independent models.[26, 27] We then proposed that the level of h-FABP protein expression in the heart was mostly related to the degree of hyperglycemia. It could be possible that the upregulation of h-FABP in the diabetic heart is the compensatory response in order to support an increase in fatty acid utilization upon lacking of glucose. Without this compensation, energy deprivation might be occurred and then leads to cardiac pathology and exercise intolerance as found in h-FABP-deficient mice.[3]

Our findings rule out the potential regulation of h-FABP protein expression in the heart. The results suggest that the expression of h-FABP in the heart is mainly regulated by the energy source availability. Hypertrophy of the heart, induced by either physiological or pathological stresses, was not involved in regulating h-FABP expression. Moreover, there was no association between h-FABP expression in the heart and myocardial dysfunction. Therefore, h-FABP expression could be another specific marker indicating abnormal energy metabolism in the heart.

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Conflict of interest: All authors report no conflicts of interest relevant to this article.

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