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Metabolic Markers of Insulin Resistance in Feline Hypertrophied Myocardium. Insulin Resistance in Heart Failure

[1,2]*Oleinikov Dmitrii Arkadievich

[1]*Almazov National Medical Research Center, IEM, St. Petersburg, Russia*
[2]*Vetetinary hospital 'Beliy Klyk', Moscow, Russia*

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Corresponding Author:
Oleinikov Dmitrii Arkadievich
Almazov National Medical Research Center, IEM, St. Petersburg, Russia
And
Vetetinary hospital 'Beliy Klyk', Moscow, Russia
Email: wolfberg.guard@gmail.com

Abstract: Investigation of myocardial tissue concentration of ATP, glucose transporters 1 and 4, pyruvate dehydrogenase, hexokinase 2, insulin receptor and adropin proteins, determining metabolic changes and possible insulin resistance in feline myocardium with hypertrophic phenotype. This is the pilot study for metabolic markers determination. Eighteen cats were studied, divided into 3 groups: Without cardiac disease (n = 5), cats with hypertrophic cardiomyopathy (n = 8), cats with chronic kidney disease and secondary myocardial hypertrophy (n = 5). Animals in the study were diagnosed for the primary disease by standard methods and algorithms. Cats were euthanized due to the end-stage chronic kidney disease, refractory heart failure or by owners' will. The myocardium samples were obtained immediately after death. Samples for the metabolic study were taken from the apical part of the left ventricular free wall and fixed in liquid nitrogen at once and were stored in -80°C refrigerator. Studied proteins concentrations were analyzed in a specialized research laboratory, using ELISA kits, provided by Cloud-Clone Corp. (USA), included: Total ATP, pyruvate dehydrogenase, hexokinase II, Adropin, insulin receptor, GLUT1 and GLUT4. In the group with HCM, we discovered that levels of ATP, pyruvate dehydrogenase and adropin were severely suppressed in comparison to healthy cats, while GLUT1 and GLUT4 did not change. The concentration of hexokinase 2 and insulin receptor proteins significantly increased. In the group of secondary myocardial hypertrophy, suppression of most studied proteins was admitted, except insulin receptor. In conclusion, we discovered metabolic remodeling and development of insulin resistance in observed diseases with hypertrophy phenotype. We observed depression of pivotal enzymes proteins, limiting energy restoration potency for cardio myocytes.

Keywords: Myocardial Metabolism, Insulin Resistance, Myocardial Hypertrophy, Feline, Hypertrophic Cardiomyopathy, Chronic Kidney Disease

Introduction

Heart failure syndrome is an aspect of primary or secondary heart disease and it is associated with decompensation, formation and activation of pathological interactions between components of neurohumoral regulation systems. This state is also characterized by myocardial energy metabolism suppression (Herrmann and Decherd JR, 1939). For example, systolic dysfunction is associated with sympathetic nervous system activation, leading to heart rate acceleration. This catecholamine-induced action provokes increased oxygen demand in the myocardium, increased Free Fatty Acids (FFA) consumption as an energy resource, ADP accumulation and negative inotropic effect. These conditions are well observed in chronic sympathetic hyperactivity in heart failure (Fukushima and Lopaschuk, 2016; Liu *et al*., 2001; Neubauer, 2007; Opie *et al*., 1979; Stanley *et al*., 1997a-b).

The main substrates for ATP formation in the myocardium are carbohydrates and FFA (Lopaschuk *et al*., 2010). Additionally, other energy resources should be mentioned: Triglycerides (TG), long-chained fatty acids,

glucose, glycogen, lactate, pyruvate, ketone bodies (acetoacetate, beta-hydroxybutyrate) and in some cases amino acids (leucine, valine, isoleucine). Listed substrates are degraded to intermediates, which then are included into the Kreb's cycle as an Acetyl-Coenzyme A (ACoA) or as a metabolic equivalent. During the utilization of these substrates, a proton generated, forming electrical potential between mitochondrial membranes and by the respiratory chain, ADP to ATP is phosphorylated (Ingwall, 2002; 2009; Taegtmeyer *et al*., 2016; Ventura-Clapier *et al*., 2004).

This dispersion in substrates for uniform energy fuel is a base for several concepts: (1) myocardial metabolism is adaptive to the whole organism state and substrates availability, it can autonomically change for relevant energy supply, however, in the heart failure this flexibility is limited; (2) myocardial metabolism is automatically regulated; all the metabolic intermediates are mediators, controlling metabolism pathways and their intensity (the Randle's cycle); (3) intermediates can be used for cell structure synthesis and, albeit, cell's are degrading, the structural components can be utilized as energy resource; (4) metabolic alterations and intermediates accumulation can damage cell's proteins and decrease the ability of the cardiomyocytes to contract; (5) myocardial metabolism is not "cell chemistry", it is a functional unit, with its own structure and mediators, which control cardiomyocyte adaptivity (Knüpfer *et al*., 2013; Taegtmeyer *et al*., 2016).

The most common cause of heart failure in cats is a Hypertrophic Cardiomyopathy (feline HCM/HCMf). This disease is characterized by left ventricular wall hypertrophy and diastolic dysfunction. Clinical and pathological aspects of this disease are very similar to humans HCM (Fox *et al*., 1995).

HCMf phenotype has a grade of phenotype variability and can be in a latent form for a long time. Furthermore, this disease can lead to heart failure development, associated with pulmonary edema, arterial thromboembolism and sudden death. In human medicine, there are more than 1500 mutations and 11 genes described, which associated with HCM. In cats, only 2 genes, associated with family HCMf in Maine coons and Ragdolls are known. Unfortunately, in other breeds, genes inducing HCMf are not investigated (Harris *et al*., 2011; Maron *et al*., 2014). Moreover, pathogenetic mechanisms responsible for hypertrophy development are unknown. There is a hypothesis, that energy depletion in Cardiomyocytes (CMC) is a common pathological component, regardless of a causing factor (Christiansen *et al*., 2015c; Crilley *et al*., 2003).

There are some diseases which provoke phenotypically similar to HCMf changes in myocardium: Hyperthyroidism, Hypertension and Chronic Kidney Disease (CKD). In this pilot work, we tried to find possible differences in some aspects of glucose metabolism in Healthy Cats (HC) myocardium, cats with hypertrophied left ventricular walls, associated with myocardium disease (HCMf) and extracardial cause (CKD).

Materials and Methods

In this study, we studied 18 cats of different sex, age and breed. All cats were patients of veterinary clinics in Saint Petersburg, Russia. Groups were formed according to the primary pathology: Cats without cardiac disease (HC, $n = 5$); cats with Hypertrophic Cardiomyopathy (HCMC, $n = 8$); cats with Chronic Kidney Disease (CKDC, $n = 5$).

Animals in the study were diagnosed for the primary disease by standard methods and algorithms (clinical findings, anamnesis, blood analyzes, radiography, echocardiography, electrocardiography, blood pressure measurement). In a group of cats without cardiac disease (HC) we included animals that had no signs of cardiac disease by clinical observation, anamnesis or by methods of echocardiography, electrocardiography. For inclusion to HCMC group, we followed standard recommendations with inclusion criteria, such as: Wall thickness, atrial size, systolic and diastolic dysfunction, mitral flow, tissue dopplerography (Häggström *et al*., 2015; Paige *et al*., 2009). Cats had a classical form of feline HCM. The group CKDC was formed by animals with terminal stage of CKD (IRIS 4), which was treated for a long period (about 1 year) with standard therapy and had secondary arterial hypertension, without inherited or acquired heart diseases or hyperthyroidism. Echocardiography showed mild left ventricular walls hypertrophy (with the accent on interventricular septum), associated with systemic hypertension. This manifestation is a part of Target-Organs Damage (TOD: Vessels, kidneys, heart, eyes, central nervous system) of systemic hypertension, which associated with CKD (Leclerc *et al*., 2017; Vickery *et al*., 2005).

Patients without signs of heart failure or left ventricular Hypertrophy (HC) in this study were euthanized due to low quality of life (most of them had the primary neurological disease). Cats from other groups were euthanized due to end-stage CKD (CKDC), refractory Heart failure (HCMC) or by owners' will. Obtaining samples was agreed with the owners.

The materials were obtained immediately after death. Samples for the metabolic study were taken from the apical part of the left ventricular free wall and fixed in liquid nitrogen at once and then were stored in -80C refrigerator.

For biochemistry analysis we used ELISA kits, provided by Cloud-Clone Corp. (USA) and included: Total ATP, Pyruvate Dehydrogenase (PDH), Hexokinase II (HX2), Adropin (Adr), GLUT1 and GLUT4, Insulin Receptor (IR). The tests were provided by the means of Almazov National Medical Research Center, IEM laboratory.

Statistical analysis was performed using commercially available software, in particular Fisher exact test and Mann-Whitney U-test by STATISTICA 7.0 software.

Results obtained from biochemical studies were used as a normal physiological state. All the comparative evaluations of the other groups were provided in comparison to HC data.

First of all, we must mention that the data about most of the parameters we studied is not presented in available articles, monographs, books. Therefore, we used data from cats without cardiac diseases as physiological state and compared these findings with information collected from mice, rat, dog and human studies.

Results

Observed ATP concentration in the HC group shows sufficient amount of macroergic phosphates for myocardium contractility energy supply (Table 1), due to the fact of absence of heart failure. This data and tissue concentrations of GLUT1 and GLUT4 (Table 2), IR (Table 3), PDH and HX2 (Table 4), ADR (Table 5), were assumed as the physiological state.

Table 1: ATP concentration in the myocardium

Group	ATP concentration, μmol/g of wet tissue
HC (*n* = 5)	6,1±0,66
HCMC (*n* = 8)	0,65±0,38*
CKDC (*n* = 5)	0,94±0,41*++

* - p≤0,01 in comparison to HC
** - p≤0,05 in comparison to HC
+ - p≤0,01 in comparison to HCM
++ - p≤0,05 in comparison to HCM

Table 2: Presence of GLUT1 and GLUT4 in the myocardium

Group	GLUT1 concentration, ng/g of wet tissue	GLUT4 concentration, ng/g of wet tissue
HC (*n* = 5)	785,28±93,21	364,91±47,63
HCMC (*n* = 8)	622,57±105,62**	407,63±26,71**
CKDC (*n* = 5)	52,32±7,00*+	147,39±39,33*+

* - p≤0,01 in comparison to HC
** - p≤0,05 in comparison to HC
+ - p≤0,01 in comparison to HCM
++ - p≤0,05 in comparison to HCM

Table 3: Insulin receptor concentration in the myocardium

Group	Insulin receptor concentration, ng/g of wet tissue
HC (*n* = 5)	1885,66±172,96
HCMC (*n* = 8)	7044,54±375,60*
CKDC (*n* = 5)	6778,36±417,64*+++

* - p≤0,01 in comparison to HC
** - p≤0,05 in comparison to HC
+ - p≤0,01 in comparison to HCM
++ - p≤0,05 in comparison to HCM
+++- p≥0,05 in comparison to HCM

Table 4: Pyruvate dehydrogenase and Hexokinase 2 concentration in the myocardium

Group	PDH concentration, ng/g of wet tissue	HX 2 concentration, ng/g of wet tissue
HC (*n* = 5)	14,22±0,54	1565,74±260,81
HCMC (*n* = 8)	3±0,65*	5720,65±1152,09*
CKDC (*n* = 5)	1,86±0,76*++	1290,55±621,64***+

* - p≤0,01 in comparison to HC
** - p≤0,05 in comparison to HC
***- p≤0,05 in comparison to HC
+ - p≤0,01 in comparison to HCM
++ - p≤0,05 in comparison to HCM
+++- p≥0,05 in comparison to HCM

Table 5: Adropin concentration in the myocardium

Group	Adropin concentration, ng/g of wet tissue
HC (*n* = 5)	7,18±1,02
HCMC (*n* = 8)	2,17±0,65*
CKDC (*n* = 5)	3,01±1,13*+++

* - p≤0,01 in comparison to HC
** - p≤0,05 in comparison to HC
***- p≤0,05 in comparison to HC
+ - p≤0,01 in comparison to HCM
++ - p≤0,05 in comparison to HCM
+++- p≥0,05 in comparison to HCM

Next, in HCMC group we found a very low concentration of ATP (Table 1) in the myocardium (HC, p≤0.01).

There is a difference in myocardial GLUT1 and GLUT4 distribution between HC and HCMC cats. In cats without heart failure, we found a high concentration of GLUT1 proteins. In Table 2 we observe, that GLUT1 and GLUT4 proteins were not significantly depressed in HCMC group (p≤0.01; p≤0.01 respectively). However, the level of insulin receptor (IR) increased dramatically (Table 3) in HCMC to HC cats (p≤0.01).

Moreover, we observed glucose utilization depression on PDH level (Table 4). Also, we admitted that due to increased HX2 proteins (Table 4), that glucose is still transported to CMC (possibly, due to GLUT1 function, because it can work in cases of insulin resistance). HX2 in HCMC is almost twofold higher than in HC cats (p≤0.01), but PDH proteins concentration was severely decreased in comparison to HC (p≤0.01). Adropin level (Table 5) in the myocardium of HCMC was significantly lower than in HC group (p≤0.01).

For comparison, we also studied non-cardiac disease with left myocardium hypertrophy. For this reason, we used data obtained from cats with CKD and secondary myocardial hypertrophy.

ATP concentration (Table 1) found in CKDC group myocardium was severely depleted. Estimated ATP was significantly lower than in HCMC group and non-significant differs from HCMC cats (p≤0.01 and p≥0.05, respectively).

In CKDC GLUT1 and GLUT4 proteins (Table 2) concentration decreased dramatically (HCMC, p≤0.01; HC, p≤0.01). While IR (Table 3) was significantly higher, than in HC cats (p≤0.01) and did not differ from HCMC cats (p≥0.05).

Changes in HX2 and PDH (Table 4) characterize significant suppression of glycolysis in the myocardium. PDH concentration in CKDC was almost 10 times lower than in HC (p≤0.01) and non-significantly differ from HCMC (p≥0.05). HX2 proteins expression were also significantly suppressed in comparison to HC and HCMC groups (p≤0.01; p≤0.01 respectively).

Adr expression presented significantly lower in comparison to HC group (p≤0.01) and did not differ from HCMC (p≥0.05).

Discussion

The obtained data from apparently healthy cats shows that myocardium has low insulin-depended source of glucose transporter (this rises a question about myocardial metabolism in cats with diabetes and low frequency of diabetic cardiomyopathy) and, possibly, normal CMC has alternative paths for carbohydrates (monocarboxylate transporter-1, GLUT12, GLUT5, fructose utilization, malate-aspartate shuttle, etc.).

In HC group we found relatively high concentration of ATP in tissue samples, moreover it was higher in comparison to the dog's myocardium (Arkadievich, 2017). Then, we discovered high proteins concentration of GLUT1 than GLUT4 (Table 2), which may be explained by the prevalence of insulin-independent glucose transport in cat's heart. Additionally, we found a great difference between these proteins in comparison with the dogs studied earlier (Arkadievich, 2017). These features may also be explained by anaerobic predomination in the feline myocardium, due to different evolutionary lifestyles.

This hypothesis is also sustained by the presence of insulin receptors proteins in the feline myocardium. In cats without heart failure, concentration of insulin receptors is considerably low. This can be an evidence of the prevalence of insulin-independent glucose consumption by the myocardium.

Glucose oxidative axis was presented in this study by two enzymes Pyruvate Dehydrogenase (PDH) and Hexokinase 2 (HX2). We defined that PDH proteins concentration is twofold higher in cats than in pigs (Siess and Wieland, 1972), which also could highlight the intensity of glucose utilization in the feline heart. The HX 2 Table 4 proteins presented in the feline myocardium were non-significantly higher than in rats myocardium (Holroyde and Trayer, 1976). This cross-species diversity rises a question about possible differences in physiology, pathophysiology and treatment stratages.

In a recent study of dependence between myocardial hypertrophy, body weight and Insulin-Like Growth Factor (IGF-1) in cats identified that animals with hypertrophied left ventricular walls had higher plasma concentrations of insulin and IGF-1, but glucose level was slightly lower than in cats without hypertrophy (Freeman *et al*., 2015). This data can show that hypertrophy of left ventricular walls in cats is associated with mild hyperinsulinemia and glucose decrease, showing the IRM development. This is also sustained by increased IGF-1 - regulator of insulin sensibility. Unfortunately, we have a lack of information in the

metabolic features of the feline myocardium, but there is a study about method's utility of mitochondrial OXPHOS estimation with different substrates (Christiansen *et al*., 2015b). Additionally, there is a study, dedicated to mitochondrial OXPHOS evaluation in feline HCM and HCM effects on Kreb's cycle enzymes activity and intensity of FFA oxidation (Christiansen *et al*., 2015c). This mosaic data helped us to find some connections between developing changes in myocardium metabolomic, associated our findings with previous information.

In HCMC group, we observed significant decrease in ATP concentration. This data supports the hypothesis of the 'lowest energy threshold' for terminal failure (Ingwall, 2009) and is consistent with the general concept of terminal heart failure pathogenesis. Also, we should admit, that ATP concentration in HCM cats was similar to the data obtained in dogs terminal HF from our previous research (Arkadievich, 2017), despite its very high concentration in the healthy myocardium. During cardiomyopathy progression and insufficiency of available sources of energy, the expression of the adaptive gene is developed, associated with CMC metabolism changes and switching to fetal genotype expression. The most remarkable process include increased proteins GLUT1 and development of insulin resistance, increased beta-Myosin Heavy Chains (beta-MHC) synthesis, switching to BB-homo isomer of creatine kinase (has more affinity to ADP and is more effective to sustain adequate ATP concentration) (Friehs *et al*., 1999; Kolwicz Jr and Tian, 2011; Paternostro *et al*., 1999; Razeghi *et al*., 2001; Schlattner *et al*., 1998; Tokarska-Schlattner *et al*., 2005; Wallimann *et al*., 1992). Associated with disease, activation of adrenergic nervous system provokes hyperinsulinemia, insulin resistance and altered GLUT4 membrane trafficking (Bonen *et al*., 1992; Richter and Hargreaves, 2013; Watt and Hargreaves, 2002).

Human studies admitted that HCM has a weak influence on the GLUT4/GLUT1 ratio, but simultaneously, a small decrease of GLUT4 and significant GLUT1 proteins expression is observed. Thus, on some stage, glucose and basal consumption is suppressed, due to GLUT1 proteins depression and increased insulin-dependent glucose utilization, associated with GLUT4. Nevertheless, during decompensation, both GLUT1 and GLUT4 proteins are dramatically depleted (Paternostro *et al*., 1999).

Increased Insulin receptor expression, found in HCMC group, could be explained by increased myocardial demand in carbohydrates (glucose) as an energy substrate, by the way, low ATP concentration shows inefficiency of this adaptation.

Thus, in HCM cats we can observe some kind of glycolytic path acceleration due to IR increased expression, but there is a block on PDH, limiting oxidative phosphorylation. This PDH block could be associated with compensatory FFA utilization, as an alternative energy substrate. However, this way leads to PDH-kinase 4 activation and depression of mitochondrial oxidation (Atherton *et al*., 2011).

Adropin is a new marker of the myocardium metabolic state, this protein can be found both in tissue homogenates and plasma. This small peptide is a regulator of lipid/carbohydrate metabolism activity most of the organism tissues including the heart. Recent data showed a correlation between this hormone plasma concentration and heart failure in human patients (Lian *et al*., 2011). Decreased Adropin expression in myocardium is an evidence of developing Insulin Resistance (IRM) in feline HCM with HF and decompensation of energy supplying processes. Human studies have confirmed that Adr is associated with AMK function and is a sign of maladaptive condition with increased death risks (Lipovka and Konhilas, 2015).

It is known that diastolic dysfunction associated with pathological myocardium hypertrophy is closely connected with Sarcoplasmic Reticulum (SR) Ca+2-ATP-ase, Na+/K+- ATP-ase and pump dysfunction. These pumps have mostly glucose-dependent energy supply. During remodeling and hypertrophy, these act as enzymes and pump becomes depleted, which leads to decreased ions flux, Ca+2 accumulation and slowed relaxation (de la Bastie *et al*., 1990; Nagai *et al*., 1989).

Studies dedicated to myocardial myosin metabolism revealed that its half-life is about 6-8 days (unlike muscle - about 30 days). Moreover, alpha-myosin isoforms are sensible to available ATP, i.e., in conditions of ATP insufficiency alpha chains are replaced by beta-Myosin Heavy Chains (beta-MHC), which are less sensitive to depleted macroergic phosphates, but less flexible and unable to contract fast (Bouvagnet *et al*., 1984; Mercadier *et al*., 1987; Tsuchimochi *et al*., 1984). In a study with hypertrophy model developed due to aorta constriction, accelerated myosin metabolism in left ventricle myocardium was admitted, causing alpha-myosin replacement for 90% with beta-MHC by the 15's day of the experiment (Kimata and Morkin, 1971). This basis can help us to bound ATP depletion, changes in glycolytic metabolism with hypertrophy progression during HCM development. Also, this information is important for understanding treatment efficiency and therapy changing according to a stage and aggressiveness of the myocardium hypertrophy. The transitory hypertrophy of left ventricle walls in cats demonstrates the previous hypothesis. This disease is of unknown etiology but is associated with myocardial edema, coronary circulation insufficiency and sympathetic nervous system hyperactivation (Novo Matos *et al*., 2018). If the signs of the disease were found in time, therapy with beta-blockers could prevent pathological remodeling to HCM phenotype (blocking beta-MHC replacement).

The data obtained in CKD group supports the hypothesis of IRM development. Human studies also admitted a high risk of heart complications due to CKD, especially in cases of the IRM and hyperinsulinemia (Kaysen, 2007; Stefanović *et al*., 2003). CKD provokes left ventricular myocardium hypertrophy, myocardial contractile dysfunction, IRM, CMC and myocardial remodeling and shift to fetal gene expression, with the accent on glycolytic myocardial metabolism (Amann and Ritz, 2001). In a long perspective, these conditions lead to alteration of glucose transport to CMC, mostly due to GLUT-system dysfunction (Paternostro *et al*., 1999). In an experiment on rats with induced chronical uremia (6 weeks) disturbances of myocardial GLUT4 concentration and development of IRM were found (Aksentijević *et al*., 2009). In our study, we do not observe increased GLUT4 proteins expression, this could be explained by a very long period of uremia in CKD cats (treatment of persistent uremia for CKD group cats was about a year, in comparison to 6 weeks in rats experiment).

Changes in GLUT1, GLUT4, IR in CKDC group show myocardial condition similar to HCM animals, with a remark to the chronic process in CKDC group. This is the reason for decreased production of all studied proteins and attendance to accelerated glycolysis through insulin-mediated pathways. Changes in HX2 and PDH in CKDC group could be explained by decreased glucose flux into CMC, PDH and FFA oxidation-dependent inhibition of HX2 proteins synthesis and microRNA HX2 inhibition by FFA metabolites (Braithwaite *et al*., 1995; Postic *et al*., 1994).

Cats with CKD could have myocardium hypertrophy primarily to decreased GLUT1 and GLUT4 (Stenbit *et al*., 2000). Histologically, animals with depleted GLUT4 expression, have pathological CMC hypertrophy, vascular sclerosis, interstitial fibrosis and this condition is similar to chronic hypertension (Buttrick *et al*., 1988; Scheuer and Buttrick, 1987). Additionally, in studies with triiodothyronine-provoked hyperthyroidism, myocardium hypertrophy, glycolysis suppression, decreased GLUT1 and GLUT4 proteins expression was admitted (Hopkins and Clarke, 1997).

Changes in HX2 and PDH in CKD cats could be explained by non-effective glycolysis and increased FFA oxidation due to the Randle cycle. In experiments with hyperthyroid animals, PDH suppression was observed as a result of PDH-kinase activation; this change is found also in hypertensive rats. In studied rats additionally, myocardial hypertrophy was admitted. During treatment with dichloroacetate (PDH-kinase inhibitor) activity of PDH was restored (Atherton *et al*., 2011). In case of CKD cats, we possibly, face the same condition.

Adropin concentration in CKDC group significantly decreased, but was similar to HCMC, this fact also shows a possible effect of insulin resistance development in cats with chronic kidney disease.

Conclusion

In conclusion, in this study, we found that primary or secondary heart disease with myocardial hypertrophy phenotype on terminal stages is associated with severe changes in energy supply systems. The data diversity shows evidence of insulin resistance development in different ways. In the “fast-developed” disease (HCM) we admitted increases in IR, GLUT4, HX2 proteins concentration, as a way of increased glucose influx. However, depleted PDH and Adr show the inability of adequate glucose utilization and decreased insulin sensitivity as a feedback reaction. In the “slow-developed” case (CKD), we discovered global suppression of studied proteins, except insulin receptor. The most notable fact is significantly decreased GLUT4 protein concentration and extremely high IR presented. This disproportion shows severe IRM and inability of long-term hypertrophied myocardium to produce enough energy. Decreased Adr concentrations are an indirect marker of decreased glucose consumption and active FFA utilization. Moreover, in this study, we can admit that decreased myocardium ATP concentration is closely connected to a terminal stage of the myocardial disease. This data is consistent with data from human medicine - ATP/phosphocreatine ratio is an independent predictor of cardiac death.

In this study, despite the fact that it has pilot profile, we found a connection between energy insufficiency in myocardium and tissue concentration of adropin. Therefore, in further studies, Adr could be a plasma biomarker of the heart failure stage and elucidate the role of IRM development in heart diseases.

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Ethics

The scheme of this study was ethically approved on clinical conference and by Research Standard department of Almazov National Medical Research Center.

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