Cholesterol-rich diet induced improvement of hemodynamic system indices in SHR

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Metabolic syndrome is the nowadays problem that arose as a result of changes in lifestyle and diet. Several of the key factors of this disorder are lipid metabolism disturbances and hypertension. However, there is little known about combined influence of this factors and their contribution to the functional changes. This work is aimed to elucidate the effects of cholesterol-rich diet on major hemodynamic indices and lipid metabolism in SHR rats. The experiments were performed in 6-month old Wistar and SHR rats. Animals were fed for 8 weeks with enriched by cholesterol (3% w/w, Sigma) standard rodent rations. Cardiohemodynamics measured in vivo by Pressure-sensitive microcatheter 2F (“Millar Instruments”, USA) introduced into left ventricle. Blood cholesterol profile was measured biochemically. The obtained data, paradoxically, signified that unlike the Wistar rats in SHR the major heart function parameters like Ejection fraction (31% increase), Stroke Volume (72% increase), Cardiac Output (69% increase) etc. improved after cholesterol diet. And at the same time SHR were more resistant to changes in blood lipid composition – they kept the same level of HDL and reached 46% lower LDL level than Wistar rats. Thus, chronic arterial hypertension reduces the sensitivity of rats to exogenous cholesterol. Combined influence of cholesterol and hypertension protects heart function in comparison to normotensive rats.

Key words: hypertension; lipid metabolism; cholesterol diet; cardiohemodynamics; spontaneously hypertensive rats.

INTRODUCTION

Many researches are indicating the great value of lipid metabolism in the field of heart pathophysiology, particularly, in cardiac failure development [1-4]. AKT/PI3K pathway is involved in high cholesterol response of the heart cells [5], and this is one of the main metabolic pathways responsible for cardiac growth, in particular hypertrophic [6]. Hypertrophy in turn is the main adaptive response of the heart to increased afterload, which can be caused by hypertension. Ho-Jin Park and colleagues [1] showed a link between lipid metabolism and parasympathetic response in chicken atrial myocytes and mouse heart that may affect normal functioning of the heart, and provide possible connection to cardiac arrhythmias development. The rhythm disorders are known to be concomitant to hypertension-induced hypertrophy [7, 8]. Also there is a connection between hypertension and lipid disorders/obesity in pathological condition known as metabolic syndrome, and these factors are pivotal in its development [9]. The purpose of our work was to investigate the combined influence of cholesterol and hypertension on hemodynamic system function and lipid metabolism.

METHODS

Animal model and experimental design
The experiments were performed in 6-month old Wistar and spontaneously hypertensive (SHR) rats. In order to induce atherogenesis, the animals were fed for 8 weeks with standard rodent rations enriched by cholesterol (3% w/w, «Sigma»,USA). The rats were divided in 4 experimental groups: 1) Wistar rats (n=16, standard diet, weight 294±22g), 2) Wistar rats (n=16, 3% cholesterol diet, weight 288±78g), 3) SHR (n=12, standard diet, weight 293±43g), 4) SHR (n=16, 3% cholesterol diet, 328±50g).
All SHR were previously tested and assured for their hypertensive state (arterial pressure over 150 mm Hg). This procedure was performed using sphygmomanometer (“HSE”, Germany). Rats were carefully placed in the cylindrical box in the way to leave their tails exposed for examination. The pressure measurement was conducted in resting state of animals.

Hemodynamic parameters examination
Heart function indices were acquired from rats, narcotized by urethane (1.5 g/kg). Pressure-sensitive microcatheter 2F (“Millar Instruments”, USA) [10] was introduced to left ventricle of the heart through the dissected left common carotid artery. After that, using provided software from Millar Instruments, the heart P-V loops were registered and analyzed. In order to obtain myocardium elasticity data, some occlusions of vena cava inferior were performed through the little incision below the metasternum. The occlusion data were acquired during direct short term squeezing of vena cava inferior and subsequent lowering of blood flow in the heart. Using provided software, a list of hemodynamic parameters was acquired: heart rate, end-systolic pressure, end-diastolic pressure, end-systolic volume of left ventricle, end-diastolic volume of left ventricle, stroke volume, cardiac output, dp/dt max and min, time constant for isovolumic relaxation (Tau), acquired by Weiss method (1976) etc. Effective arterial elastance was calculated as a ratio of end-systolic pressure to stroke volume [7]. Pressure-volume interrelations were analyzed using PVAN 3.6 software (“Millar Instruments”, USA). Conventional volume units from raw data were converted to volume units (µl) using calibration equation (slope 20.25X RVU-Intercept 29.05). For calibration purposes, the catheter was immersed in calibration cuvette with several known diameter and volume cylindrical holes. Cuvette was filled with heparin stabilized rat blood.

Blood lipid profile determination
Concentration of cholesterol, triglycerides, lipoproteins of high, low and very low density were investigated in rat blood using biochemical analyzer Bio System A25 (“Bio-Systems” S.A., Spain). Atherogenic index was calculated by the next equation (TC-HDL)/HDL.

Statistical analysis
Statistical significance of differences between experimental groups (pretested by Levenes test for homoscedasticity, and by Kolmogorova-Smirnova normality test) was tested by ANOVA with post-hoc multiple comparisons using Games-Howell test.

RESULTS AND DISCUSSION
The hypothesis behind this work was that the cholesterol-rich diet was expected to worsen hemodynamic indices in Wistar rats and especially in SHR as they already have pathological process at work. Indeed, the cholesterol-rich diet changed the pumping function of the heart of Wistar rats in comparison to standard diet by a number of indices (Fig.1, 2, Table): stroke volume (20% decrease), ejection fraction (1% increase), cardiac output (33% decrease), stroke work (13% decrease), maximal power (23% decrease).

However, the pumping function of the heart of SHR rats with cholesterol-rich diet was changed in a different way by some indices (Fig. 1, 2, Table): stroke volume (72% increase), ejection fraction (31% increase), cardiac output (69% increase), stroke work (77% increase), maximal power (59% increase).

Systolic function was evaluated by the next set of indices, describing difference between Wistar rats on cholesterol-rich versus standard diet (Fig. 3, Table): heart rate (11% decrease), end-systolic volume (33% decrease), end-systolic pressure (34% increase), maximal speed of pressure change dp/dt max (22% decrease), end-systolic maximum volume elasticity (Emax) (99% increase), maximal speed of volume change dV/dt max (36% decrease), volume at the point of maximal speed of pressure change (30%
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Fig. 1. Effect of cholesterol diet (3%) on stroke volume and ejection fraction parameters of Wistar and SHR rats.
* statistically significant difference comparing to Wistar rats on standard diet (P<0.05)
** statistically significant difference comparing to SHR on standard diet (P<0.05)

Fig. 2. Effect of cholesterol diet (3%) on cardiac output and stroke work of Wistar and SHR rats.
* statistically significant difference comparing to Wistar rats on standard diet (P<0.05)
** statistically significant difference comparing to SHR on standard diet (P<0.05)
Cardiohemodynamic parameters of Wistar rats and SHR while on standard or cholesterol diet (M±SE).

| Parameter                              | Wistar                          | Wistar cholesterol diet | SHR                          | SHR cholesterol diet |
|----------------------------------------|---------------------------------|-------------------------|------------------------------|----------------------|
| Heart rate, bpm                         | 363.4 ± 18.1                    | 320.4 ± 17.8*           | 308.6 ± 10.4*                | 288.2 ± 14.7* **     |
| End-systolic volume, µL                 | 493.9 ± 37.9                    | 329.6 ± 18.6*           | 434.3 ± 58.2*                | 530.4 ± 64.05* **    |
| End-diastolic volume, µL                | 682.4 ± 40.9                    | 493.1 ± 65.7*           | 460.4 ± 45.9*                | 614.4 ± 75.2* **     |
| Maximal speed of pressure change dP/dt max, mmHg/s | 1.3 ± 1.7                       | 2.6 ± 1.7*              | 5.7 ± 2.7*                   | 5.2 ± 1.1*           |
| End-diastolic pressure, mmHg            | 9337.3 ± 855.9                  | 11484.2 ± 464.5*        | 7169.5 ± 314.2*              | 6524.3 ± 1046.3* **  |
| dP/dt_min, mmHg/s                      | -9221.5 ± 981.1                 | -6579.6 ± 806*          | -6810.9 ± 315.2*             | -6353.9 ± 962.2* **  |
| Tau_w, ms                              | 7.8 ± 0.4                       | 9.6 ± 1.4*              | 10.08 ± 1.02*                | 10.6 ± 0.8* **       |

* statistically significant difference comparing to Wistar on standard diet (P<0.05)
** statistically significant difference comparing to SHR on standard diet (P<0.05).

decrease). Systolic function indices describing SHR rats with cholesterol-rich versus standard diet difference: heart rate (6% decrease), end-systolic volume (22% increase), end-systolic pressure (13% decrease), maximal speed of pressure change dP/dt max (9% decrease), end-systolic left ventricular maximum volume elasticity (Emax) (37% decrease), maximal speed of volume change dV/dt max (74% increase), volume at the point of maximal speed of press-

Fig.3. Effect of cholesterol diet (3%) on end-systolic pressure and end-systolic left ventricular maximum volume elasticity (Emax) of Wistar and SHR rats.
* statistically significant difference comparing to Wistar rats on standard diet (P<0.05)
** statistically significant difference comparing to SHR on standard diet (P<0.05)
Diastolic ability of the heart of Wistar rats on cholesterol versus standard diet was evaluated by the next indices: end-diastolic volume (27% decrease), end-diastolic pressure (93% increase), isovolumic left ventricular relaxation time constant Tau evaluated by Weiss (23% increase) method, minimal speed of pressure change dP/dt min (28% decrease), minimal speed of volume change dV/dt min (15% decrease) (Table). Same set of indices was evaluated in SHR rats on cholesterol versus standard diet difference: end-diastolic volume (33% increase), end-diastolic pressure (8% decrease), isovolumic left ventricular relaxation time constant Tau evaluated by Weiss (6% increase) and Glantz (2% increase) methods, minimal speed of pressure change dP/dt min (6% decrease), minimal speed of volume change dV/dt min (93% increase) (Table).

Another significant index in the course of our experiment was arterial elastance, which was 2.3 times increased in Wistar rats with cholesterol-rich comparing to standard diet. But in SHR groups cholesterol-rich diet results in 47% decrease of arterial elastance (Table).

**Blood lipid profile**

In the experimental groups, cholesterol-rich diet effect was quite predictable, and the worsening of blood lipids was well expected. But, what was not so obvious, that spontaneously hypertensive rats were more tolerant to cholesterol diet than Wistar rats comparing to their respective groups on standard diet.

Blood lipid profile in Wistar rats on cholesterol-rich diet was different from those on standard diet: total cholesterol (84% increase), cholesterol of high-density lipoproteins (26% increase) and low density lipoproteins (more than 6 times increase) (Fig.5.). Same indices in SHR rats with cholesterol-rich diet were also different comparing to standard diet fed SHR: total cholesterol (81% increase), cholesterol of high-density lipoproteins (37% increase) and low density lipoproteins (3.9 times increase) (Fig.5.). There were no statistically significant differences in triglycerides, cholesterol of very low-density lipoproteins levels and atherogenic index between all groups. Though comparing mean levels it can be pointed that SHR triglycerides (SHRs were 34% higher than Wistar rats on standard diet, and 25% - on cholesterol-rich diet) were higher comparing to Wistar rats and there were little to no effect on cholesterol-rich diet on this parameters.

Thus, acquired data signified that cholesterol-rich diet induced shift of blood lipid fraction in both SHR and Wistar rats. The data may evidence that SHR rats were less susceptible to negative effects of cholesterol-rich diet on blood lipid levels, as their LDL levels after cholesterol-rich diet were significantly lower while HDL kept almost at the same concentra-

![Fig.4. Effect of cholesterol diet (3%) on Arterial Elastance of Wistar and SHR rats.](image)

* statistically significant difference comparing to Wistar rats on standard diet (P<0.05)
** statistically significant difference comparing to SHR on standard diet (P<0.05)
tion comparing to Wistar rats. These two parameters are important for the known index of atherogenesis development probability, and in Wistar rats with cholesterol-rich diet atherogenic index was higher (22% increase) than in respective SHR group. But we believe that in case of complex pathological process where there is the combined influence of several pathological factors (hypertension and high cholesterol diet) the meaning of this index could be not so much straightforward and histological researches must be conducted before any viable statement can be concluded. Nevertheless such differences in lipid profile are an effect of some changes in

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**Fig.5. Effect of cholesterol diet (3%) on blood total cholesterol, cholesterol of high-density and low density lipoproteins in Wistar and SHR rats.**

* statistically significant difference comparing to Wistar rats on standard diet (P<0.05)
** statistically significant difference comparing to SHR on standard diet (P<0.05)
SHR rats due to their hypertensive status [11, 12]. Such changes can be connected to understanding of how the excess amounts of lipids can be utilized. And the difference may be the result of some unconventional mechanisms activation in SHR, causing steatosis for example.

Also we can say that, paradoxically, cholesterol-rich diet affects hemodynamic function of Wistar rats and SHR differently, by worsening the state of the first one and by substantially improving the latter. Explanation to these unexpected results in our opinion is mainly associated with drastically improved Arterial Elastance in SHR rats, lowering afterload this way and then naturally followed by improvement of heart functions. This parameter can be connected to the blood vessels state, and taking into account the shift of blood lipid profile, we can assume the development of cholesterol-induced changes in arterial wall of SHR rats.

Thus, according to data acquired we can conclude that cholesterol-rich diet improved cardiac function parameters, in particular Arterial Elastance, in SHR comparing to Wistar rats. Also, SHRs were more resistant to deleterious effects of cholesterol-rich diet on their LDL level in blood.

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ПОКРАЩЕННЯ ГЕМОДИНАМИЧНИХ ПОКАЗАТЕЛЕЙ У КРЫС СО СПОНТАННОЙ ГИПЕРТЕНЗИЕЙ ПОД ДЕЙСТВІЕМ ХОЛЕСТЕРИНОВОЇ DIЕТY

Целью этой работы является определение эффектов холестериновой диеты на все основные гемодинамические показатели и липидный метаболизм у спонтанной гипертензивных крыс. Эксперименты проводились на 6-месячных самцах крыс линии Вистар и со спонтанной гипертензией. Животные находились на обогащенной холестерином (3%) стандартной диете грызунов на протяжении 8 нед. Показатели кардиогемодинамики измеряли in vivo с помощью чувствительных к давлению и объёму микрокатетеров 2F (“Millar Instruments”, США), которые вводили в полость левого желудочка. Холестериновые фракции крови определялись биохимически. Полученные результаты, парадоксально, свидетельствуют об улучшении под действием холестериновой диеты параметров функции сердца крыс с гипертензией, в частности: фракции выброса (увеличилась на 31%), ударного объёма (увеличился на 72%), минутного объёма сердца (увеличился на 69%) и т.д. В то же время крысы с гипертензией были более стойкими к изменениям в составе холестериновых фракций крови, в частности однократное с крысами линии Вистар содержание ЛПВП, но на 46% более низкое ЛПНП. Таким образом, артериальная гипертензия уменьшала чувствительность
рыс к экзогенному холестерину. Совместное влияние холестерина и гипертензии оказывало протективное действие на функцию сердца в сравнении с крысами с нормальным давлением.

Ключевые слова: гипертензия; липидный метаболизм; холестериновая диета; кардиогемодинамика; крысы со спонтанной гипертензией.

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