Liver Dysfunction Induced by Bile Duct Ligation and Galactosamine Injection Alters Cardiac Protein Synthesis

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Liver disease has been shown to affect the cardiovascular system and may influence cardiac protein metabolism. This hypothesis was tested by measuring rates of cardiac protein synthesis in 2 models of liver disease in rats. The study consisted of 5 groups— group 1: control, injected with saline and fed ad libitum; group 2: acute liver injury, by dosage with 400 mg/kg galactosamine; group 3: injected with saline and pair-fed to group 2; group 4: chronic liver disease, using bile duct ligation; and group 5: sham-operated and pair-fed to group 4. Rates of cardiac protein synthesis were measured using the flooding dose technique. After 1 week, galactosamine injection caused the following cardiac changes, i.e. group (2) versus (3): an increased RNA content, RNA/DNA ratio, and RNA/protein ratio. However, there was no change in DNA or protein content, or protein/DNA ratio. There was an increase in the fractional rate of protein synthesis, and the absolute synthesis rate. Cellular efficiency was increased, but RNA activity remained unchanged. Comparison of groups 4 and 5 showed that bile duct ligation caused no change in any parameters measured. Although comparison of the ad libitum–fed group 1 with the bile duct ligation group 4 showed reduced cardiac weight, protein, and RNA content, with decreased right ventricular absolute synthesis rates; this was also seen in the pair-fed group 5, suggesting that these effects were due solely to reduced oral intake. Thus, although galactosamine-induced acute liver injury caused marked changes in cardiac biochemistry, bile duct ligation per se did not. This study also illustrates the importance of including a pair-fed group.

HEMODYNAMIC abnormalities have been shown to occur at rest in over a third of patients with liver cirrhosis.[1] Cirrhosis is associated with a hyperdynamic circulation, which manifests as a reduced peripheral vascular resistance with an increased cardiac output. Although hyperdynamic circulation has been demonstrated at rest, abnormal cardiac function becomes more pronounced when subjected to additional functional loads.[2] Echocardiographic and hemodynamic studies have shown impaired systolic and diastolic function due to liver disease.[3,4]

With the advent of new therapeutic interventions for cirrhotic patients, concern has arisen that these procedures may impose additional stress on the heart unmasking subclinical cardiac disease. Heart failure accounts for between 7% and 21% of deaths following liver transplantation.[5,6] Other studies have also reported on the prevalence of heart failure among patients following various portosystemic shunt procedures.[7-10]

The cause of impaired cardiac function in nonalcoholic liver disease is uncertain. Postmortem examinations have shown cardiac hypertrophy and ventricular dilatation, as well as ultrastructural changes on histology.[11] Animal models of nonalcoholic liver disease have shown increased circulating levels of catecholamines, causing β-adrenoceptor desensitization and downregulation.[12,13] Expression and function of G-proteins are also reduced, further implicating this pathway.[14] Fluidity of plasma membranes in the heart is reduced due to an increase in membrane cholesterol content which also affects β-receptor functioning.[15] Circulating endotoxins and cytokines such as tumour necrosis factor and the interleukins may also depress cardiac function by stimulating nitric oxide production.[16,17]

Liver disease perturbs hepatic synthesis of insulin-like growth factors (IGFs) and the IGF-binding proteins (IGFBP) that control cell growth and protein synthesis.[18] Animal models of liver disease have been shown to impair rates of growth, reduce skeletal muscle protein content, and decrease heart weight.[19] However, the possibility that cardiac protein metabolism may be affected by nonalcoholic liver disease has not yet been investigated. This study aimed to investigate the effects of different types of liver disease on cardiac protein synthesis. To discover whether any of the effects of liver disease were mediated by malnutrition, rats were pair-fed to the liver disease groups and a control group fed ad libitum was also included.

MATERIALS AND METHODS

Treatment of Animals

Male Wistar rats, aged 30 days with a mean weight of 69 g, were chosen for the study as the youngest animals upon which surgery could be performed successfully. The rats were housed in a humidified, temperature-controlled environment on a 12-hour light/12-hour dark cycle. They were all fed standard laboratory chow and had free access to water. The 5 groups were as follows: group 1 was injected with saline intraperitoneally and food was available ad libitum; these rats served as a control group. Group 2 was injected with 400 mg/kg galactosamine intraperitoneally to produce an acute liver injury, and food was available ad libitum. Group 3 was injected with saline intraperitoneally and pair-fed to group 2. Pair-feeding was achieved by recording the intake of food by the experimental group, and providing the same quantity to the pair-fed group at the beginning of following day. Group 4 underwent bile duct ligation under anaesthesia to create a model of cholestatic liver disease, and food was available ad libitum. Group 5 was sham-operated and pair-fed to group 4. All animals were treated in accordance with the ethical guidelines provided by the university at which the study was conducted.

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Galactosamine injection and bile duct ligation were used as they are among the most established models of liver disease.[18] Other well-known models of liver disease such as intra-abdominal abcess or turpentine injection were not used due to additional effects of sepsis and direct organ toxicity. Galactosamine injection acts as a model of acute hepatitis, similar to that caused by viral infection or paracetamol poisoning, causing hepatic focal necrosis, ballooning, and inflammation.[24] However, bile duct ligation causes a rise in bilirubin and changes in liver function tests in association with histological changes similar to those that occur in chronic cholestatic liver disease, providing a model of biliary cirrhosis or biliary atresia.[25] Therefore, the use of these 2 models allows comparison of the effects of acute hepatocellular damage with the chronic effects of cholestatic liver disease on the heart.

Processing of Hearts

After 1 week, rates of protein synthesis were measured with a flooding dose of [^{3}H]phenylalanine (injected at a dose of 150 mmol/L, 1 mL/100 g body weight, intravenously) to label the intracellular and extracellular free amino acid pools. Processing of cardiac tissue for phenylalanine specific radioactivities have been described previously.[20-22] All steps, including homogenization, were carried out at between 0 and 4°C and all centrifugations were performed at 2,000 × *g* for 10 minutes, unless otherwise stated.

After the rats were killed, hearts were removed and separated into left and right ventricular portions and weighed. Mixed heart data were calculated using data for left and right ventricular portions. For analysis of RNA, and synthesis of mixed cardiac proteins, whole hearts were homogenized in ice-cold water and portions of heart homogenate containing approximately 200 to 400 mg tissue were immediately precipitated with perchloric acid to a final concentration of 0.2 mol/L. After centrifugation, the acid supernatant was decanted and neutralized with saturated tripotassium citrate, and a 5-mL aliquot incubated overnight with phenylalanine decarboxylase (pH 6.3, 52°C) for conversion of phenylalanine to 2-phenylethylamine, to obtain the specific radioactivity of the free amino acid in cardiac homogenate.[20] The cardiac protein pellet was then "washed" with 12 to 14 mL of 0.2-mol/L perchloric acid and the pellet digested in 10 mL of 0.3-mol/L NaOH, at 37.5°C for 1 hour. An aliquot (2 mL) was removed for estimation of protein by the biuret reaction.[23] The protein was reprecipitated by addition of 1.78 mL of 2-mol/L perchloric acid and RNA measured in the acid supernatant after centrifugation.[23] The protein pellet was then washed a further 6 to 8 times in 12 to 14 mL of 0.2-mol/L perchloric acid to remove traces of free phenylalanine and hydrolyzed in 6 mol/L HCl (105°C, 36 hours). The hydrolysate was dried in vacuo (over solid NaOH and P_2O_5), suspended in 3 mL buffer (sodium citrate, pH 6.3, 1.5 mol/L) and incubated with phenylalanine decarboxylase (52°C, 12 hours) to obtain the specific radioactivity of phenylalanine in cardiac protein after extraction of 2-phenylethylamine.[20]

Calculation of Protein Synthesis Rates

Fractional rates of protein synthesis (defined as the percentage of tissue protein renewed each day by synthesis, ie, k_s) was calculated from the formula:

$$k_s = \frac{S_b \times 100}{S_i \times t}, (\%/d) \qquad (A)$$

where "S_b" and "S_i" were the specific radioactivities of phenylalanine (dpm/nmol) in protein hydrolysates and free phenylalanine in acid

Table 1. Heart Weight And Content

	Ad Libitum–Fed (group 1)	Galactosamine (group 2)	Pair-Fed (group 3)	Bile Duct–Ligated (group 4)	Pair-Fed (group 5)
Wet weight (mg)					
Mixed	385 ± 10	404 ± 11	373 ± 80	333 ± 16	309 ± 10
LV	323 ± 9	331 ± 8	309 ± 8	282 ± 14*	257 ± 9*
RV	61.6 ± 3.4	72.2 ± 4.7*	63.5 ± 3.4	50.5 ± 3.0*	50.7 ± 3.2*
LV/RV ratio	5.33 ± 0.30	4.70 ± 0.27	4.94 ± 0.28	5.65 ± 0.26	5.03 ± 0.28
Protein content (mg per heart)					
Mixed	42.2 ± 1.2	43.0 ± 1.1	41.1 ± 0.6	36.3 ± 1.6*	34.6 ± 1.2*
LV	35.2 ± 1.0	35.1 ± 1.0	33.9 ± 0.7	30.6 ± 1.3*	28.6 ± 0.9*
RV	7.01 ± 0.40	7.90 ± 0.47	7.22 ± 0.33	5.77 ± 0.34*	5.80 ± 0.30*
LV/RV ratio	5.11 ± 0.29	4.56 ± 0.29	4.75 ± 0.27	5.34 ± 0.19	4.84 ± 0.16
RNA content (mg per heart)					
Mixed	0.783 ± 0.029	0.824 ± 0.027†	0.736 ± 0.022*	0.652 ± 0.037*	0.639 ± 0.036*
LV	0.608 ± 0.024	0.620 ± 0.014*	0.553 ± 0.021*	0.503 ± 0.026*	0.484 ± 0.023*
RV	0.175 ± 0.093	0.204 ± 0.017	0.182 ± 0.013	0.149 ± 0.013	0.148 ± 0.014
LV/RV ratio	3.53 ± 0.22	3.18 ± 0.25	3.12 ± 0.29	3.47 ± 0.21	3.17 ± 0.17
DNA content (mg per heart)					
Mixed	2.25 ± 0.07	2.28 ± 0.04	2.21 ± 0.05	1.96 ± 0.10*	1.83 ± 0.08*
LV	1.88 ± 0.06	1.86 ± 0.05	1.83 ± 0.06	1.65 ± 0.09*	1.51 ± 0.06*
RV	0.366 ± 0.023	0.419 ± 0.021†	0.376 ± 0.015	0.311 ± 0.020	0.318 ± 0.017
LV/RV ratio	5.26 ± 0.34	4.53 ± 0.30	4.91 ± 0.29	5.36 ± 0.28	4.68 ± 0.21

NOTE. All data are presented as mean ± SEM of 5 to 8 observations. Differences between means were assessed by Student's *t* test using the pooled estimate of variance.

*Significant difference ($P < .05$) between ad libitum controls and the other groups.

†Significant difference ($P < .05$) between the experimental groups and their respective pair-fed groups.

Abbreviations: LV, left ventricle; RV, right ventricle.

Table 2. Derived Parameters

	Ad Libitum–Fed (group 1)	Galactosamine (group 2)	Pair-Fed (group 3)	Bile Duct–Ligated (group 4)	Pair-Fed (group 5)
RNA/DNA ratio (mg/mg)					
Mixed	0.348 ± 0.006	0.362 ± 0.010†	0.334 ± 0.008	0.332 ± 0.005	0.349 ± 0.010
LV	0.323 ± 0.005	0.335 ± 0.007†	0.302 ± 0.006*	0.306 ± 0.004	0.321 ± 0.010
RV	0.483 ± 0.021	0.481 ± 0.0196	0.484 ± 0.026	0.477 ± 0.022	0.461 ± 0.022
LV/RV ratio	0.675 ± 0.026	0.701 ± 0.021	0.631 ± 0.030	0.651 ± 0.034	0.680 ± 0.040
RNA/protein ratio (mg/g)					
Mixed	18.5 ± 0.3	19.1 ± 0.5†	17.9 ± 0.30*	17.9 ± 0.4*	18.5 ± 0.5
LV	17.2 ± 0.3	17.7 ± 0.3†	16.3 ± 0.3*	16.4 ± 0.2*	16.9 ± 0.5
RV	25.0 ± 0.7	25.6 ± 0.9	25.2 ± 1.0	25.7 ± 1.7	25.2 ± 1.1
LV/RV ratio	0.692 ± 0.020	0.695 ± 0.020	0.652 ± 0.030	0.653 ± 0.035	0.654 ± 0.030
Protein/DNA ratio (mg/mg)					
Mixed	18.8 ± 0.2	18.9 ± 0.2	18.7 ± 0.3	18.6 ± 0.2	18.9 ± 0.2
LV	18.7 ± 0.2	18.9 ± 0.2	18.6 ± 0.3	18.6 ± 0.2	19.0 ± 0.2
RV	19.3 ± 0.4	18.8 ± 0.3	19.2 ± 0.3	18.7 ± 0.8	18.3 ± 0.3
LV/RV ratio	0.975 ± 0.017	1.01 ± 0.01	0.97 ± 0.01	1.01 ± 0.04	1.04 ± 0.02

NOTE. All data are presented as mean ± SEM of 5 to 8 observations. Differences between means were assessed by Student's *t* test using the pooled estimate of variance.

*Significant difference ($P < .05$) between ad libitum controls and the other groups.

†Significant difference ($P < .05$) between the experimental groups and their respective pair-fed groups.

supernatants of cardiac homogenates, respectively, and "t" was the period (in days) between injection of isotope and immersion of the heart into ice/water mixture.

The amount of protein synthesis per unit RNA (RNA activity) or DNA (cellular efficiency) was calculated from the formula:

$$k_{RNA} \text{ or } k_{DNA} = \frac{k_s \times 10}{(\text{mg nucleic acid/g protein})},$$

(in mg protein/d/mg nucleic acid) (B)

The absolute rate of protein synthesis (defined as the total amount of protein synthesised each day, ie, V_s) was calculated from the protein content and fractional synthesis rate, using the formula:

$$V_s = \frac{\text{total protein content} \times V_s}{100}, \text{(mg/d)} \quad \text{(C)}$$

Statistics

Data are presented as the mean ± SEM of 5 to 8 findings in each group. Differences between means were assessed using least significant differences (LSD) incorporating the pooled estimate of variance. *P* values greater than .05 were considered not significant (NS).

RESULTS

Compared to its pair-fed group (group 3), galactosamine (group 2) caused an increase in whole heart RNA content, RNA/DNA ratio, and RNA/protein ratio (all $P < .025$; Tables 1 and 2). However, there was no change in DNA or protein content, or protein/DNA ratio (Tables 1 and 2). The galactosamine group showed an increase in both the whole heart fractional synthesis rate from 12.6 ± 0.9%/d to 14.7 ± 0.6%/d ($P < .025$), and an increase in the whole heart absolute synthesis rate from 5.22 ± 0.43 mg/d to 6.33 ± 0.34 mg/d ($P < .025$; Table 3). Cellular efficiency was also increased ($P < .025$), but RNA activity remained unchanged in the galactosamine group compared to pair-fed rats (Table 3). The galactosamine group showed an increase in the right ventricular weight and absolute synthesis rate compared to the ad libitum–fed group (both $P < 0.05$; Tables 1 and 3). The galactosamine-injected group (group 2) showed an increase in right ventricular weight and right ventricular absolute synthesis rate compared with the ad libitum group (group 1). However, protein and RNA contents remained unchanged, with no change in the fractional rate of protein synthesis.

Comparing groups 4 and 5 shows that there was no difference between the bile duct–ligated and pair-fed groups in any of the parameters measured. Comparison of the bile duct–ligated group (group 4) with the ad libitum group (group 1) showed a reduction in heart weight and protein, RNA, and DNA contents in the bile duct–ligated group (all $P < .05$; Table 1). There was a decrease in left ventricular RNA/protein ratio, with no change in the RNA/DNA ratio (Table 2). These changes were accompanied by a fall in the right ventricular absolute synthesis rate from 0.99 ± 0.07 to 0.73 ± 0.08 ($P < .05$). In the bile duct ligation group there were also increased left/right ventricular ratios for fractional synthesis rate, RNA activities, and DNA efficiencies compared with the ad libitum–fed group (all $P < .05$; Table 3).

DISCUSSION

Animal models of liver disease have been shown to impair rates of growth, reduce skeletal muscle protein content, and decrease heart weight.[19] Impaired cardiac function has also been demonstrated in patients with liver disease.[3,4] With the advent of new therapeutic interventions for cirrhotic patients, concern has arisen that these procedures may impose additional stress on the heart unmasking subclinical cardiac disease. Heart failure accounts for up to 21% of deaths following liver transplantation.[5,6] However, the cause of impaired cardiac function in non-alcoholic liver disease is uncertain. This study aimed to

Table 3. Protein Synthesis Rates

	Ad Libitum–Fed (group 1)	Galactosamine (group 2)	Pair-Fed (group 3)	Bile Duct–Ligated (group 4)	Pair-Fed (group 5)
Fractional synthesis rate (k_s) (%/d)					
Mixed	13.1 ± 0.3	14.7 ± 0.6†	12.6 ± 0.9	12.7 ± 0.5	12.1 ± 1.1
LV	12.9 ± 0.2	14.2 ± 0.5†	12.6 ± 0.9	12.7 ± 0.6	12.8 ± 1.0
RV	14.9 ± 1.8	15.7 ± 0.7†	13.0 ± 1.0	12.5 ± 0.7	11.9 ± 1.4
LV/RV ratio	0.91 ± 0.06	0.91 ± 0.02	0.97 ± 0.03	1.03 ± 0.02*	1.04 ± 0.04*
Absolute synthesis rate (V_s) (mg/d)					
Mixed	5.49 ± 0.23	6.33 ± 0.34†	5.22 ± 0.43	4.65 ± 0.37	4.21 ± 0.47*
LV	4.54 ± 0.22	5.00 ± 0.25	4.28 ± 0.37	3.98 ± 0.35	3.48 ± 0.36*
RV	0.99 ± 0.07	1.25 ± 0.12*†	0.94 ± 0.09	0.73 ± 0.08*	0.72 ± 0.11
LV/RV ratio	4.67 ± 0.34	4.18 ± 0.33	4.64 ± 0.33	5.36 ± 0.27	5.02 ± 0.36
RNA activity (k_{RNA})					
Mixed	7.00 ± 0.15	7.66 ± 0.24	7.06 ± 0.42	7.09 ± 0.25	6.50 ± 0.42
LV	7.43 ± 0.18	8.04 ± 0.27	7.67 ± 0.43	7.72 ± 0.29	7.12 ± 0.45
RV	5.90 ± 0.80	6.13 ± 0.23	5.16 ± 0.40	4.92 ± 0.31	4.56 ± 0.43*
LV/RV ratio	1.33 ± 0.13	1.32 ± 0.04	1.50 ± 0.07	1.63 ± 0.08*	1.59 ± 0.08*
Cellular efficiency (k_{DNA})					
Mixed	2.46 ± 0.05	2.78 ± 0.13†	2.36 ± 0.16	2.36 ± 0.07	2.27 ± 0.18
LV	2.41 ± 0.04	2.69 ± 0.10†	2.32 ± 0.15	2.36 ± 0.07	2.29 ± 0.18
RV	2.89 ± 0.36	2.95 ± 0.17	2.49 ± 0.22	2.33 ± 0.17	2.18 ± 0.23*
LV/RV ratio	0.88 ± 0.08	0.92 ± 0.02	0.94 ± 0.03	1.02 ± 0.05*	1.07 ± 0.05*

NOTE. All data are presented as mean ± SEM of 5 to 8 observations. Differences between means were assessed by Student's *t* test using the pooled estimate of variance.

*Significant difference ($P < .05$) between ad libitum controls and the other groups.

†Significant difference ($P < .05$) between the experimental groups and their respective pair-fed groups.

investigate the effects of different types of liver disease on cardiac protein synthesis.

The Effect of Bile Duct Ligation

Compared with the pair-fed group, bile duct ligation caused no change in any parameters measured. There was no alterations in protein, RNA, or DNA content, or any change in protein synthesis. Compared to the ad libitum–fed group, bile duct ligation caused a reduction in the heart weight, and RNA and protein content by a mechanism that did not involve a decrease in the fractional rate of protein synthesis. This suggests an increase in cardiac protein degradation. As the pair-fed group showed very similar changes, this suggests that any effect of bile duct ligation on cardiac protein metabolism was through decreased food intake. This illustrates the importance of including a pair-fed group, as it is well recognized that altered nutrition can affect protein metabolism.

These effects are consistent with those of reduced nutrition, which have been demonstrated previously.[21,26] Malnutrition, diarrhea, ethanol consumption, and cancer have all been shown to affect protein metabolism in the heart, causing ultrastructural changes visible on microscopy.[21,26-29] Previous studies have also shown that malnutrition can adversely affect cardiac function.[30] These data suggest that reduced oral intake may contribute to cardiac pathology in cholestatic liver disease. This has clinical implications, in that correction of any nutritional defect may be beneficial for cardiac function.

The Effect of Galactosamine

Comparison of the galactosamine model of acute liver injury with its pair-fed group, ie, groups 2 and 3, showed there was an increase in the fractional rate of cardiac protein synthesis. The RNA activity (rate of protein synthesis per unit RNA) was not significantly altered, but the RNA content was increased. However, despite a 17% increase in the fractional synthesis rate, there was no change in the heart weight or protein content, suggesting a concomitant increase in proteolysis. These changes in cardiac protein synthesis may be an adaptive change to hemodynamic alterations that occur in acute liver injury, or as a result of altered circulating humoural factors.

Conclusion

Cirrhotic cardiomyopathy is a poorly characterized condition, and more work may provide novel strategies for reducing the mortality due to heart failure following surgical interventions for liver disease. These results showed no effect of cholestatic liver disease on the heart other than through reduced food intake. This suggests nutritional status may have an important influence over cardiac protein metabolism in cholestatic liver disease. The increased cardiac protein synthesis caused by acute hepatocellular injury is likely due to alterations in circulating hormonal factors. Derangement of thyroid function and the IGF axis has been shown to differ between acute hepatocellular injury and cholestatic liver disease, and may contribute to this.

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