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Regional Distribution of Total Lipids, Free Fatty Acids and Free Carnitine in Human Heart

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The concentration of total lipids, free fatty acids and free carnitine in seven different regions of human ventricular myocardium has been studied. Statistically significant differences were found in the concentration of these compunds in the studied regions, with a similar behaviour for free carnitine and free fatty acids (their highest concentrations were found in the lower zones of the left ventricle). The results obtained support the theory of a different metabolic requirement in relation to the intensity of muscular contractile activity.

Key words: Free fatty acids, Free carnitine, Total lipids, Regional distribution in heart.

Few papers in recent years have been devoted to the study of variations in the chemical composition of cardiac lipids and their metabolic intermediates in experimental models in relation to the hypoxic/anoxic processes (1, 15, 16). Some studies have ascertained variations in the cardiac levels of free fatty acids (5, 8, 19) and free carnitine (4, 16, 17) during myocardial ischemia.

Carnitine is the carrier required to transport free fatty acids from the cytosol to the mitochondrial matrix for β-oxidation (3, 9, 13). Thus in a tissue that depends on fat as an important souce of fuel, the concentration of free carnitine plays an important role in its metabolism.

It is not clear, however, if there is a homogeneous distribution of these compounds in the heart and different concentration-values could be obtained depending on the sampling zones.

In the absence of bibliography about the distribution of these compounds in the human heart, the aims of this study were to determine their concentrations in seven different regions of heart in order to find the possible «map» of distribution of these compounds.

The knowledge of these regional differences could be useful for the latter study of metabolic changes in the human heart after an ischemic process.

* A quien debe enviarse la correspondencia

Materials and Methods

Hearts were obtained from 68 corpses during medico-legal autopsy performance according to normal procedures. Causes of death were both violent (multiple traumatism, hanging, etc) and non-violent (cerebral haemorrhage, pulmonary embolism, pneumonia, etc) in aetiology. Sex distribution and age of all the cases studied were as following: 46 males with an average age of 52.2 years (range = 14-84 years) and 22 females with an average age of 57.0 years (range = 6-83 years). Hearts were dissected in seven different regions: Anterosuperior zone from the left ventricle (LV_{a-s}); Anteroinferior zone from the left ventricle (LV_{a-i}); Posterosuperior zone from the left ventricle (LV_{p-s}); Posteroinferior zone from the left ventricle (LV_{p-i}); Superior zone from the septum interventricular (S_s); Inferior zone from the septum interventricular (S_i); and Superior zone from the right ventricle (RV_s).

Total lipids were extracted according to the BLIGH and DYER method (2) with the addition of 4 ml of methanol, 4 ml of chloroform and 2 ml of saline solution (0.88 %) per gramme of tissue, with fifteen strokes of an all-glass Potter-Elvehjem homogenizer after every addition. The homogenate obtained was then centrifuged for 10 minutes at 4,000 r.p.m. Afterwards, the lower phase was weighed and redissolved with 0.5 ml of a benzene: ethanol solution (4:1, v:v).

Free fatty acid concentrations were determined according to LOWRY and TINSLEY (11), and free carnitine concentrations by the PEARSON *et al.* technique (14). All the measures were made with a Beckman Spectrophotometer Model 25.

Statistical analysis of results was made by use of the Student's t-test for paired data to compare differences between different zones. Differences in variables were considered significant when P was 0.05 or less. In addition, tests of correlation and lineal regression were made to compare the free fatty acids, free carnitine and total lipids levels with the postmortem interval.

Results

Table I summarises mean values ± S.E.M. for concentrations of total lipids, free fatty acids and free carnitine in the different cardiac regions, as well as the results from the Student's t-test for these compound concentrations among the different heart zones.

In relation to sex, there were only significant differences ($p<0.05$) for free fatty acids, free carnitine and total lipids concentrations in the superior zone from the right ventricle (RV_s) (the highest free fatty acids and total lipid values were found in females and the highest free carnitine concentration in males.

Correlation studies between free fatty acids values for different myocardial regions and the postmortem interval are shown in table II. Table III shows the results from correlation studies between free fatty acids and free carnitine.

Discussion

The first problem was the choice of a reliable essay procedure for the isolation and analysis of free fatty acids. Quantitative determination of these compounds can be carried out by chromatographic methods or by clororimetric analysis.

The determination of free fatty acids by gas-liquid chromatography (18-20) provides information about all the fatty acid species, but a troublesome isolation (by thin layer chromatography) and cumbersome derivatization of the isoflated free fatty acids are required. Nevertheless, chromatografic techniques were tested, but the results obtained were not reliable (accuracy = 46.3-62.5 % and precision = 56.41-168.75 %, depending on the different fatty acids).

Table I. *Total lipids (mg/g w.t.), free fatty acids (μg/g w.t.) and free carnitine (nmol/g w.t.) values in myocardial tissue in the different regions studied (upper), and results from the Student's t test for paired data for these compounds among the different zones of heart (lower).*

Values are mean ± S.E.M. In parenthesis number of determinations. (LV_{a-s}) Anterosuperior zone from the left ventricle; (LV_{a-i}) Anteroinferior zone from the left ventricle; (LV_{p-s}) Posterosuperior zone from the left ventricle; (LV_{p-i}) Posteroinferior zone from the left ventricle; (S_s) Superior zone from the septum interventricular; (S_i) Inferior zone from the septum interventricular; and (RV_s) Superior zone from the right ventricle.

ZONES	TOTAL LIPIDS	FREE FATTY ACIDS	FREE CARNITINE
LV_{a-s}	(66) 20.72 ± 4.75	(66) 13.07 ± 1.21	(68) 129.45 ± 6.22
LV_{a-i}	(63) 58.72 ± 4.43	(63) 22.18 ± 2.35	(55) 159.90 ± 11.73
LV_{p-s}	(66) 30.88 ± 1.70	(66) 14.59 ± 1.28	(68) 138.28 ± 7.58
LV_{p-i}	(63) 53.79 ± 4.58	(63) 23.42 ± 2.54	(60) 160.32 ± 9.82
S_s	(66) 30.10 ± 1.58	(66) 13.08 ± 1.11	(65) 124.04 ± 6.33
S_i	(63) 53.68 ± 4.06	(63) 21.97 ± 2.51–	(57) 144.39 ± 8.07
RV_s	(65) 69.38 ± 4.83	(65) 14.14 ± 1.35	(57) 113.88 ± 6.16

	TOTAL LIPIDS						FREE FATTY ACIDS						FREE CARNITINE					
	LV a-i	LV p-s	LV p-i	S s	S i	RV s	LV a-i	LV p-s	LV p-i	S s	S i	RV s	LV a-i	LV p-s	LV p-i	S s	S i	RV s
LV a-s	***	**	**	*	***	***	***	**	***	N.S.	***	N.S.	**	N.S.	***	N.S.	*	N.S.
LV a-i	—	***	N.S.	***	N.S.	*	—	***	*	***	N.S.	***	—	**	N.S.	**	N.S.	**
LV p-s	—	—	***	N.S.	***	***	—	—	***	**	*	N.S.	—	—	*	**	N.S.	*
LV p-i	—	—	—	***	N.S.	N.S.	—	—	—	***	N.S.	***	—	—	—	***	*	***
S s	—	—	—	—	***	***	—	—	—	—	***	N.S.	—	—	—	—	*	N.S.
S i	—	—	—	—	—	***	—	—	—	—	—	***	—	—	—	—	—	***

(***) $p<0.01$; (**) $p<0.01$; (*) $p<0.05$.

Table II. *Correlation studies between free fatty acid values for different myocardial regions and the postmortem interval.*
Range of postmortem intervals = 5-40 h. Average of postmortem intervals = 17.75 ± 7.34 h. d.f. = degree freedom. Abbreviations as in Table I.

ZONE	r	d.f.	
LV a-s	0.391	64	$p < 0.01$
LV a-i	0.161	61	N.S.
LV p-s	0.328	64	$p < 0.05$
LV p-i	0.583	63	$p < 0.01$
S s	0.361	64	$p < 0.01$
S i	0.278	61	$p < 0.05$
RV s	0.263	63	$p < 0.05$

Therefore, free fatty acid concentrations were determined by a colorimetric technique (11) as it had a good analitical reliability (accuracy = 103.3 % and precision = 2.7 %).

The samples studied were obtained from corpses during the medico-legal autopsy performance, as mentioned above, and the postmortem interval could introduce interferences due to the postmortem autolysis. In the present study, postmortem intervals were similar in all the cases (17.75 ± 7.34 hours) and, since this factor acted on the whole heart in the same way, it did not cause differences among cardiac regions in any one case.

Although several authors have described that serum fatty acids are one of the most stable postmortem compounds (7, 12), a statistical significant increase of free fatty acid levels in myocardial tissue has been found in relation to the postmortem interval (table II). No significant relation was found for free carnitine or total lipids in relation to that parameter.

In relation to age parameter (average age: 53.7 years, range, 6 to 84 years), a significant increase ($p<0.05$) was found only for total lipid values. In addition to these age-related concentrations, total lipids are also related to the lipidic store on the heart, which is dependent on the general metabolic state.

Total lipids do not have a direct metabolic function in a healthy heart, although they are more an expresion of a depot biding to an excess of fat than energetic fuels.

The initial hypothesis was that zones with highest metabolic requirements would have the highest free carnitine concentrations. Free fatty acids are a fuel in emergency biological situations such as anoxia or hypoxia, and they are mobilized only in these exceptional situations (8, 10, 13).

The highest free carnitine concentrations were found in the left ventricle, and the lowest in the right ventricle, with significant differences between the upper and the lower zones (table I). As to the left ventricle, the lowest values also correspond to its upper portion, where fewer muscle fibers are found.

Free fatty acids show a similar behaviour to free carnitine (table I). Correlation studies between these two parameters show significant results (table III), since both have complementary physiologic roles, as stated before.

The present results show that the various regions of the heart appear to vary in their free fatty acid and free carnitine concentrations and that the differences found in the lower cardiac zones might suggest a higher metabolic activity related to a bigger abundance of muscular fibers and contractile activity.

Table III. *Correlation studies between free fatty acids and free carnitine.*
d.f. = degree freedom. Abbreviations as in Table I.

ZONE	r	d.f.	
LV a-s	0.340	63	$p < 0.01$
LV a-i	0.418	49	$p < 0.01$
LV p-s	0.352	62	$p < 0.01$
LV p-i	0.354	54	$p < 0.01$
S s	0.224	60	N.S.
S i	0.198	51	N.S.
RV s	0.138	52	N.S.

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Resumen

Se estudia la concentración de lípidos totales, ácidos grasos libres y carnitina libre en siete diferentes zonas de miocardio ventricular humano. Se encuentran diferencias estadísticamente significativas en las concentraciones de estos compuestos en las distintas zonas estudiadas, con un comportamiento similar para la carnitina libre y los ácidos grasos libres (los valores más altos de estos compuestos se obtuvieron en las porciones inferiores del ventrículo izquierdo). Estos resultados refuerzan el concepto de la existencia de unos diferentes requerimientos metabólicos relacionados con el grado de actividad muscular contráctil.

Palabras clave: Acidos grasos libres, Carnitina libre, Lípidos totales, Distribución regional en corazón.

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