TECHNICAL NOTE

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Regional Study of Free Fatty Acids and Free Carnitine Behavior in Cardiac Tissue in Relation to Different Causes of Death

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ABSTRACT: The determination of free fatty acids (FFA) and free carnitine (FC) in seven different regions of the human heart has been done. The hearts used were classified into six groups according to the cause of death. The results show that these two parameters are useless in ascertaining the cause of death, although they can provide useful postmortem information about the duration of the agonal process.

KEYWORDS: pathology and biology, free fatty acids, free carnitine, cardiovascular system, agonal process, myocardial injury, thanatochemistry

The myocardial tissue uses free fatty acids (FFA) in addition to aerobic glycolysis in emergency biological situations (such as anoxia or hypoxia).

Experimental studies on animals have demonstrated that in these situations there is an accumulation of FFA in the injured tissue [*1-5*].

The FFA metabolization is carried out in the mitochondria through the β-oxidation process. Carnitine is the carrier required to transport FFA from the cytosol to the mitochondrial matrix for β-oxidation. Thus, in a tissue that depends on fat as an important source of fuel, the concentration of free carnitine (FC) plays an important role in the metabolism of that tissue.

During myocardial ischemia, carnitine tissue levels are depleted, with the resultant intracellular accumulation of FFA and fatty acid intermediates [*6-9*].

In the absence of a bibliography about the behavior of these two compounds in cadavers, the aims of this study were to determine their behavior in several causes of death to find their possible usefulness in solving the diagnostic problems from the forensic science viewpoint.

This work has been done at the Cátedra de Medicina Legal of the University of Granada, Spain. Received for publication 19 Oct. 1987; accepted for publication 27 Jan. 1988.

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Material and Methods

Hearts were obtained from 77 corpses during autopsy according to normal autopsy procedures, and 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).

The samples were grouped in relation to the different causes of death according to their physiopathological similarity:

Group 1: myocardial infarction,
Group 2: hanging,
Group 3: death by cerebral injury,
Group 4: multiple trauma,
Group 5: other natural deaths, and
Group 6: other violent deaths.

Total lipids were extracted according to the Bligh and Dyer method [*10*] with the addition of 4 mL of methanol, 4 mL of chloroform, and 2 mL of saline solution (0.88%) per gram of tissue, with 15 strokes of an all-glass Potter-Elvehjem homogenizer after every addition. The homogenate obtained was then centrifuged for 10 min at 4000 rpm. Afterwards, the lower phase was dried under a stream of nitrogen and the remaining lipid was 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 [*12*]. All the measures were made with a Beckman Spectrophotometer Model 25.

The statistical study of the results was carried out with Anova 1 to compare differences between different causes of death. In addition, we made tests of correlation and lineal regression to compare the evolution to FFA level with those of FC.

Results

The different results by zones and causes of death are shown in Tables 1 and 2.

The statistical study has been carried out only in the two cardiac zones with the highest clinical incidence of myocardial infarction (posteroinferior zone from the left ventricle and inferior zone from the septum interventricular). We have found significant values for FC myocardial tissue levels among the causes of death shown in Table 3.

Correlation studies between FFA values for different myocardial regions and the postmortem interval are shown in Table 4. Table 5 shows the results from correlation studies between FFA and FC.

Discussion

We have found the highest FFA values in Groups 5 and 6, "natural deaths" and "other violent deaths," and the lowest in Groups 4 and 1, "multiple trauma" and "myocardial infarction."

We have found significant statistical differences ($p \leq 0.05$) for the FFA concentrations from the LV p-i between Group 5, "other natural deaths" and Groups 1 and 4, "myocardial

TABLE 1—*Free fatty acids values (μg/g) in myocardial tissue in relation to the various causes of death.*

	Myocardial Infarction			Hanging			Deaths by Cerebral Injury			Multiple Trauma			Other Natural Deaths			Other Violent Deaths		
Zones	*n*	*x*	S.D.	*n*	*x*	S.D.	*n*	*x*	S.D.	*n*	*x*	S.D.	*n*	*x*	S.D.	*n*	*x*	S.D.
LV a-s	20	10.49	6.77	11	15.80	11.60	11	14.47	12.70	8	7.18	2.62	10	15.92	10.31	6	14.57	15.10
LV a-i	21	15.71	12.28	8	24.82	28.06	11	25.34	20.15	8	10.68	8.16	9	33.46	29.66	6	22.99	13.76
LV p-s	20	11.62	8.83	11	18.85	13.30	11	15.44	12.62	8	8.46	4.45	10	17.89	8.45	6	15.29	14.93
LV p-i	21	15.49	9.99	8	26.76	32.81	11	27.128	24.31	8	11.53	9.59	8	34.25	25.99	7	25.37	18.45
S s	21	9.99	5.35	10	18.75	15.84	11	13.54	9.86	8	6.63	3.61	10	16.11	8.99	10	31.11	12.05
S i	21	17.24	13.28	7	36.72	47.09	11	19.94	14.95	8	10.32	4.56	9	27.61	26.31	7	20.03	13.37
RV s	20	10.76	5.88	10	15.86	13.52	11	15.23	9.89	8	6.91	3.60	10	21.05	11.62	6	16.82	20.67
Mean values	144	13.10	3.14	65	17.32	5.57	77	18.73	5.54	56	8.82	2.01	66	21.92	7.16	48	19.25	4.18

TABLE 2—*Free carnitine values (nmol/g) in myocardial tissue in relation to the various causes of death.*

	Myocardial Infarction			Hanging			Deaths by Cerebral Injury			Multiple Trauma			Other Natural Deaths			Other Violent Deaths		
Zones	*n*	*x*	S.D.	*n*	*x*	S.D.	*n*	*x*	S.D.	*n*	*x*	S.D.	*n*	*x*	S.D.	*n*	*x*	S.D.
LV a-s	20	118.14	46.03	11	166.01	39.113	11	180.76	106.88	9	110.77	44.01	11	107.26	48.21	6	93.74	23.38
LV a-i	18	163.88	108.83	6	218.59	90.65	10	269.65	205.21	8	115.52	39.28	9	102.33	54.86	4	89.45	23.05
LV p-s	20	124.41	62.42	11	165.211	34.61	11	209.93	139.07	9	132.57	61.37	10	100.30	46.38	5	97.24	31.23
LV p-i	19	145.08	67.46	8	242.27	106.01	11	224.92	112.33	8	138.55	60.93	8	115.08	74.90	6	96.02	43.52
S s	20	114.56	48.17	11	157.06	31.83	11	191.40	124.04	9	119.86	36.43	10	92.08	38.80	4	69.25	26.83
S i	18	141.86	57.71	8	206.79	75.85	10	204.61	110.47	8	124.19	65.98	7	94.24	40.89	6	94.70	14.68
RV s	16	107.57	41.41	9	145.52	62.05	9	132.74	52.24	9	113.57	62.60	9	103.60	36.32	5	80.30	24.63
Mean values	131	130.79	20.10	64	185.92	36.44	73	202.00	41.90	60	122.15	10.20	64	102.12	7.78	36	87.67	10.31

TABLE 3—*Statistical significant values for free carnitine levels among various causes of death for different myocardial regions.*

Zone	Cause of Death	Myocardial Infarction	Multiple Trauma	Other Natural Deaths	Other Violent Deaths
LV p-i	Hanging	$p \leq 0.01$	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.01$
	Deaths by cerebral injury	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.01$
S i	Hanging	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.05$
	Deaths by cerebral injury	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.01$	$p \leq 0.01$

TABLE 4—*Correlation studies between free fatty acids values for different myocardial regions and the postmortem interval.*[a]

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

[a]Range of postmortem intervals = 5 − 40 h. Average of postmortem interval = 17.75 ± 7.34 h.

TABLE 5—*Correlation studies between free fatty acids and free carnitine.*

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

infarction" and "multiple trauma," and for the FFA concentrations from the S i between Group 2, "hangings," and the two former groups.

There is no agreement between our initial hypothesis and the results obtained. Nevertheless, there is an explanation regarding myocardial infarction; that is, the lipid increase in the cardiac tissue starts between 60 min and 6 h after the beginning of impaired blood supply to the myocardium [*13–16*]. After necrosis, there are three successives phases:

(1) alarm reaction and mobilization of triglicerides [*14*],

(2) decrease of cellular fatty acids absorption [*17–19*], and

(3) increase in the metabolism of the fatty acids to avoid their harmful action on myocardial cells [*5,6,15*].

As we said earlier, we have found the lowest FFA values in Groups 4 and 1, "multiple trauma" and "myocardial infarction." These findings are different from those obtained by other authors in animals [*3,4,5,20,21*]. Our results could be explained in the light of possible interferences of postmortem interval, but we cannot apply this explanation because the data are similar for all of the groups studied. Although several authors have described that fatty acids are one of the most stable postmortem compounds [*22,23*], we have found an increase of FFA levels in relation to the postmortem interval (Table 4).

On the other hand, our results could be related to the length and intensity of agonal suffering. During agony, there is a mobilization of catecholamines [*24–26*] and other biogenic amines (for example, 5-hydroxy tryptamine) [*27,28*] that cause activation of lipases with an increase in serum FFA levels. In processes with a great intensity of agonal suffering (for example, asphyxia), we could find a serum FFA rise that, in an initial phase, is placed in tissues with the highest metabolic demand (brain, heart) for their later metabolization.

There is a close relationship between cause of death and agonal suffering because both are unique processes that lead to the death.

To establish different patterns of agony, we differentiated between three groups [*27*]:

(1) death without survival time (immediate death, for example, massive craniocerebral trauma),

(2) death with a brief survival time (but with a long interval to start the biological response to aggression, for example, multiple trauma), and

(3) death with a long survival time from the initial process ("fading away") with exhaustion of the biological resources (for example, sepsis).

These patterns could be explained in that in violent deaths with enough survival time, we found the highest FFA values, while myocardial infarctions with scarce survival time (demonstrated by histochemical—formazan test[3] and microscopic techniques—hematoxylin eosin stain and acridine orange method), those were the lowest. In this former group, death occurs suddenly, and, although there is an initial rise of serum FFA, there is not time enough for the increase of these compounds in myocardial tissue.

Correlation studies between FFA and FC show significant results (Table 5), which have an easy explanation because both have a complementary physiopathologic role, as we have explained earlier.

We have found the lowest FC values in Group 6, "other violent deaths," followed by Group 5, "other natural deaths." On the whole, FC has a similar behavior to FFA, and consequently, all the above considerations are useful. Nevertheless, our results show that the two parameters studied are useless to ascertain the cause of death.

Our results show the difficulties of interpretation of postmortem biochemical parameters. It is necessary to evaluate carefully all the possible related factors (agony, postmortem interval, cause of death), and, in this way, we can complete and improve the morphological findings.

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[3]The formazan test is a macroscopic technique which demonstrates the activity of succinic dehydrogenase (SDH) in the heart by using neotetrazolium chloride salt. It is a useful indicator of anoxic/ischemic damage when demonstrable absence or reduction in enzyme content is apparent before any gross or classical histological abnormalities are visible.

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