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Effect of 24-hours Fast on Aspartate Transaminase Activities in the Organs of the Rat

X. Remesar, Ll. Arola [1], A. Palou [2] and M. Alemany [2, 3]

Fisiología General
Facultad de Biología
Universidad de Barcelona
Barcelona-7

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The aspartate transaminase activity of liver, kidney, brain, stomach, small intestine, striated muscle, skin and adipose tissue of fed and 24-hour fasted rats has been studied. The activity level of the enzyme is very much alike in most organs when expressed per unit of protein or DNA weight, in accordance with the general metabolic role of this enzyme. The activity of skin and adipose tissue is considerably lower than that of the other studied organs. A 24-hour fast induced significant increases in the activity of all organs except intestine and stomach.

Aspartate transaminase (E.C. 2.6.1.1) is the most widespread transaminase, as it links the glutamate metabolism with that of aspartate and oxalacetate. The enzyme controls the key pathway for alpha-amino groups transfer from amino acids — through glutamate — to aspartate, for utilization in urea and purine nucleotide cycles; as well as providing a means of ammonia transport between cytoplasmic and mitochondrial spaces. Aspartate transaminase has been found and well characterized in practically all organs and species studied (5, 8, 16, 17, 19). Most of the enzyme found in skeletal muscle and heart belongs to the cytoplasmic or soluble isoenzyme, while liver and kidney contain the mitochondrial enzyme in a higher proportion (3, 8).

The available data on the effects of fasting upon aspartate transaminase activity in mammalian organs is considerably sparse. The effect of fasting upon liver and muscle aspartate transaminase is not significant for the first 24 hours (9) being noticeable in the liver only after 48 hours (9).

Present addresses:

(1) Bioquímica. Facultad de Química. Universidad de Barcelona. Tarragona (Spain).

(2) Bioquímica. Facultad de Ciencias. Universidad de Palma de Mallorca. Palma de Mallorca, Baleares (Spain).

(3) To whom all correspondence must be sent.

The aim of the present study was to determine the effects of a 24-hour period of fasting upon the aspartate transaminase activity of the different tissues of the rat, as a way to obtain information of the overall amino acid metabolism in the fasted rat.

Materials and Methods

Albino virgin Wistar female rats, weighing initially 193 ± 6 g were used. The animals were kept under standard conditions (2). The fasted group had all food (rat chow pellets) removed 24 hours before sacrifice. All animals were killed by decapitation with guillotine at the beginning of a light cycle. Samples of their liver, kidney, brain, stomach, small intestine, skin, lumbar adipose tissue and hind leg striated muscle were dissected, blotted, cleaned and homogenized in chilled modified Krebs-Ringer bicarbonate buffer (2). Homogenates were coarsely passed through nylon-hose in order to remove pieces of tissue that could clog micropippetes; they were directly used for enzyme determinations. Aspartate transaminase was measured spectrophotometrically (4, 10) with aspartate as substrate.

Aliquots of the homogenates were used for protein (12, 18) and DNA (13) estimation. Muscle mass was stimated after AROLA *et al.* (1). The adipose tissue mass in the rats was assumed to be approximately 20 % of their body weight as they had a mean 66 % of fat in adipose tissue (15) and Wistar rats fed rat chow had a mean 13.6 % of body fat (15). This factor was used for the estimative value given in the expression of results per unit of rat weight.

Four different expressions of enzyme activity are shown for a better interpretation of results, as previously discused (2).

Results

A 24-hour fast induced a mean 9.7 ± 0.4 % body weight loss in the fasted group.

Table I. *Aspartate transaminase activity in organs of fed and 24 hours fasted rats.* All values are the mean ± s.e.m. of 6 to 8 different animals. Significance of the differences versus fed controls: * = $p < 0.05$.

Organ	Feeding status	Activity in μkat/100 g of tissue	Activity in μkat/g of protein	Activity in μkat/g of DNA	Activity in μkat/100 g of animal
Liver	fed	166.3 ± 9.4	9.64 ± 0.54	257.4 ± 14	6.97 ± 0.38
	fasted	243.3 ± 9.6 *	12.1 ± 0.39 *	238.6 ± 9.4	8.36 ± 0.32 *
Intestine	fed	32.8 ± 2.4	2.96 ± 0.21	135.0 ± 9.8	0.77 ± 0.068
	fasted	35.2 ± 1.4	2.50 ± 0.09	106.7 ± 4.2 *	0.78 ± 0.043
Stomach	fed	52.0 ± 4.6	5.21 ± 0.45	236.4 ± 21	0.25 ± 0.024
	fasted	52.9 ± 1.6	6.34 ± 0.19	311.2 ± 9.0 *	0.30 ± 0.013
Kidney	fed	134.9 ± 6.7	10.3 ± 0.51	367.6 ± 18	1.08 ± 0.065
	fasted	154.2 ± 5.6	10.3 ± 0.35	335.0 ± 11	1.37 ± 0.070 *
Striated muscle	fed	101.8 ± 7.1	9.54 ± 0.66	702.1 ± 49	44.9 ± 3.1
	fasted	124.3 ± 6.1 *	10.2 ± 0.52	887.9 ± 42 *	54.8 ± 2.7 *
Adipose tissue	fed	1.9 ± 0.3	0.81 ± 0.12	27.1 ± 4.0	0.38
	fasted	3.2 ± 0.5 *	0.85 ± 0.07	33.0 ± 5.1	0.64
Brain	fed	127.9 ± 7.5	11.9 ± 0.69	706.6 ± 42	1.06 ± 0.081
	fasted	154.4 ± 6.4	11.0 ± 0.48	712.7 ± 31	1.38 ± 0.093
Skin	fed	4.9 ± 0.9	1.15 ± 0.21	29.0 ± 5.3	0.85 ± 0.17
	fasted	8.1 ± 0.3 *	1.08 ± 0.04	33.8 ± 1.2	1.48 ± 0.015 *

Table I shows the aspartate transaminase activities observed in the different organs of adult fed and fasted rats. The data are expressed in μkat or nkat per 100 g of tissue weight, per gram of protein or DNA and per 100 g of animal weight (activity present in the whole organ referred to a uniform rat weight).

When the data are expressed per unit of tissue weight, the highest aspartate transaminase activity is found in the liver, followed by the kidneys, brain, muscle, stomach, intestine, skin and finally, adipose tissue with a fraction of activity about 1/83th of that of liver. Per unit of protein weight the pattern is similar, but now the highest activity corresponds to brain, closely followed by kidney and liver; the adipose tissue activity is now 1/12th of that of brain. Per unit of DNA weight the only important change is that muscle activity approaches nearly that of brain. The activity per unit of animal weight, however, shows a maximum for muscle, followed by liver, kidneys and brain, and then skin, intestine, adipose tissue and stomach.

Fasting induces some changes in the general pattern, as there are significant increases in the aspartate transaminase activity in liver, muscle, brain, skin and adipose tissue. The general pattern of distribution of aspartate transaminase in the different tissues is similar to that found in the fed animals, with highest activities for liver, brain and muscle and lower for skin and adipose tissue.

Discussion

The aspartate transaminase activity of liver, kidney, brain, muscle and even stomach is found in a close range of magnitude, indicating that this enzyme is mainly related to overall metabolic functions and not to a very specific pathway, as with other transaminases.

The range of activities found in liver, muscle, brain and kidney is similar to that found by ZIMMERMAN *et al.* (19). The data found here for liver, kidney and striated muscle are about 20 % lower per unit of tissue weight than those described by HERZFELD and GREENGARD (8), however the brain enzyme level is in the same range. The general increase with fasting in the aspartate transaminase activity observed in muscle, liver, kidney, brain, skin and adipose tissue seems not to be related with the increase in a single isoenzyme form, as both liver-kidney-brain and muscle — the first with predominance of the mitochondrial enzyme (3, 8) and muscle mainly cytoplasmic (3, 8) — show practically the same rate of increase in enzyme activity. There are also no differences between the splanchnic organs and the peripheral ones, as the first group (liver, kidney, stomach and small intestine) had a mean combined activity increase per unit of animal weight of about 20 %, and the second group (muscle, adipose tissue, skin and brain) had practically the same increase. The composite activity of the peripheral organs cited is roughly 5-6 times higher that of the combined splanchnic organs.

In this work we have found significant differences in the whole liver aspartate transaminase of 24-hour fasted rats with regard to the controls, thus detecting earlier the increase found by other authors (9) after a 48-hour fasting. The increase in aspartate transaminase activity of the liver must be seen under the light of the direct relationship between overall aspartate transaminase activity and gluconeogenic ability of the liver, as postulated by several authors (7, 14), as this enzyme provides most of the oxalacetate used in the liver (and kidney) gluconeogenic pathway (6); thus, the increase in liver and kidney activity can be partly related with an enhanced gluconeogenesis. The increase in the peripheral organs must be considered mainly as a mechanism for ammonia disposal in connection with amino

acid catabolism, as the aspartate synthesized can be used by the purine nucleotide cycle (11), thus providing a means for mineralization of alpha-amino nitrogen in muscle.

Resumen

Se determina la actividad aspartato transaminasa de hígado, riñón, cerebro, estómago, intestino delgado, músculo estriado, piel y tejido adiposo lumbar de ratas control y sometidas a un ayuno de 24 horas. Los niveles de actividad de este enzima son muy similares en la mayoría de los órganos estudiados cuando se expresan por unidad de peso de proteína o de ADN, lo que concuerda con el papel metabólico general de este enzima. Las actividades de la piel y del tejido adiposo son mucho más bajas que las de los restantes órganos estudiados. Un ayuno de 24 horas induce un aumento significativo de la actividad aspartato transaminasa de todos los órganos estudiados a excepción del intestino delgado y del estómago.

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