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Effect of Chronic Feeding of Remington's Diet and Fasting on Rat Plasma Composition

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Rats fed on Remington's low iodine diet (supplemented with iodine) and on rat chow were compared as to their plasma composition. There are significant differences in plasma glycerol, aceto-acetate, β-hydroxybutyrate and in several amino acid concentrations, mainly lysine and tryptophan. 24 hours of fasting lowered the differences between the groups, but some aspects remained unchanged, i.e. the low lysine and tryptophan concentrations in Remington diet fed rats. The use of the Remington diet therefore, provoked a functional lack of these amino acids, even when their concentration in the diet was higher.

The utilization of low iodine diets for the study of chronic effects of thyroid hormones in experimental animals is considerably widespread. One of the most favored formulations is that described by REMINGTON (14), based mainly in corn flour and dried yeast, with a very low iodine content. In order to assess the possible nutritional artifacts induced by chronic utilization of this diet on growing experimental animals, the following experimental setup was designed.

Materials and Methods

Weaned albino Wistar rats were used; two groups were randomly selected and kept in collective wire-mesh-bottomed cages in a light (12 h on/12 off) and temperature (22 ± 1°C) controlled animal room. The control group was fed laboratory rat chow pellet (Panlab, Barcelona, Spain), the percentual composition of which is given in table I. The experimental group was fed with Remington's low iodine diet sup-

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plemented with 1 ppm of iodine in the form of potassium iodate; the diet was prepared freshly each day; its composition can also be seen in table I. Animals of the first group had free access to tap water and those of the second one had distilled water to drink. All food and water were available in excess at any time.

On day 39th, half of the controls had all food removed at the onset of the light cycle; the same was done with the Remington-fed rats. Exactly 24 h later all the animals were beheaded with guillotine and their blood was received into dry heparinized beakers. Thus four groups of animals were studied: fed and 24 h fasted controls and fed and 24 h fasted rats fed Remington's formulation.

The hematocrit value of the blood was estimated by centrifugation. Plasma proteins were determined with the FOLIN phenol reagent (10), plasma urea was determined with the BERTHELOT method (6), and plasma free fatty acids were determined with the method of FALHOLT *et al.* (5). Plasma aliquots were deproteinized with perchloric acid, neutralized with potassium bicarbonate, and used for the estimation of glucose (9), lactate (8), glycerol (4), aceto-acetate (11) and beta-hydroxybutyrate (16). Aliquots of plasma were deproteinized with cold acetone (2) and used for the determination of individual amino acids (3).

Statistical comparisons between means were done with the Student's *t* test.

Results and Discussion

Table II shows the hematocrit value and plasma components of the fed and 24-h fasted controls and animals subjected to the Remington diet. There are no significant differences in the hematocrit value. In the fasted state, plasma protein concentrations are significantly lower in the Remington diet-fed rats than in the controls.

Table I. *Percentual composition of the pellet fed to the control group and of the Remington formulation* *

	Remington (%)	Panlab chow (%)
Fat	2.97	2.54
Protein (Kjeldahl)	30.2	21.1
Starch	45.7	40.6
Sugar (as glucose)	0.5	4.55
Protein amino acids:	(percent of the protein fraction)	
Lysine	3.54	2.27
Methionine	1.69	1.06
Cystine	1.76	0.94
Tryptophan	0.53	0.39

(*) Analysis carried on by LIC Laboratories, Barcelona, Spain.

There are no differences in the glucose, free fatty acids, urea and total, essential and non essential amino acids in the controls versus Remington's diet rats, both in the fed and fasted state. However, there exists a significant difference in both fed versus fasted states for glucose, free fatty acids, urea and total and non essential amino acids. There is, also, a significant increase in the concentrations of glycerol, aceto-acetate and beta-hydroxybutyrate in the Remington diet fed animals versus controls; this difference disappears in the fasted state, with values in both cases significantly higher than those of the fed animals. This seems to be related to a differential treatment of the lipidic foodstuffs by the pellet-fed and the Remington diet fed animals, with higher circulating levels of ketone bodies and lower (but not significant) values of free fatty acids.

Figure 1 hows the individual values of the plasma amino acids of fed and fasted rats subjected to both types of diet. In the fed state, the Remington diet rats showed significantly higher concentrations of alanine, proline and valine, and had significantly lower concentrations of as-

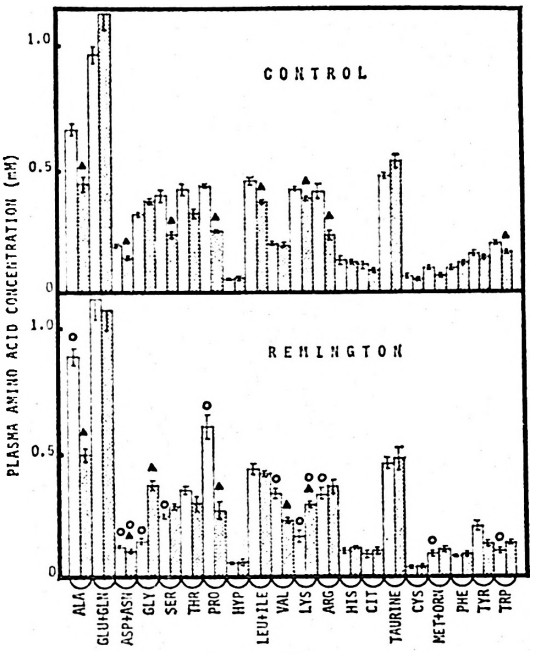

Fig. 1. *Plasma amino acid concentrations in controls and rats fed the Remington diet, both in the fed and 24-hours fasted state.*
All columns are the mean ± s.e.m. of 5 different animals. The shadowed columns correspond to the fasted animals and the white ones to the controls. The symbols used are the same as in table II.

partate plus asparagine, glycine, serine, lysine and tryptophan. In the fasted state the differences are lower; increases are observed only for arginine and methionine plus ornithine, and only decrease the level of aspartate plus asparagine, lysine and tryptophan. The low concentrations of lysine and tryptophan found in the plasma of Remington diet rats is not related to a lack of availability of these essential amino acids, as there is plenty of them in the Remington's diet with levels of lysine and tryptophan higher than in the rat chow pellets used as control. Thus the lower concentrations are not a consequence of lack of availability, but probably a consequence of impaired assimilation or utilization due to some other nutritional deficiency.

The metabolic effects provoked by fasting upon the plasma amino acid homeostasis are also different in both groups. Fasting induces a considerable decrease in the concentration of several amino acids (12, 13), mainly as a consequence of their utilization in gluconeogenesis or in other energetic pathways (1, 7, 15). The drop in amino acid concentrations after 24 hours of fasting is better seen in some

Table II. *Blood and plasma composition of controls and Remington-fed animals at 0 and 24 hours of fasting.*
All values are mean ± S.E.M. of 5 different animals. Significance of the differences versus controls: O = $p < 0.05$. Significance of the differences versus fed animals: ▲ = $p < 0.05$.

	CONTROLS		REMINGTON-FED	
	Fed	Fasted	Fed	Fasted
Hematocrit value (%)	44.7±1.1	46.6±1.4	47.0±0.9	48.5±1.9
Plasma proteins (g/l)	69.0±1.8	71.9±1.1	66.1±1.1	67.5±0.5 O
Plasma glucose (mM)	8.72±0.17	5.65±0.18 ▲	8.14±0.25	5.71±0.39 ▲
Plasma glycerol (μM)	153±2	274±18 ▲	179±22 O	157±17
Plasma free fatty acids (μM)	200±20	447±83 ▲	159±22	559±74 ▲
Plasma aceto-acetate (μM)	38.1±2.8	363±20 ▲	67.3±6.2 O	533±24 ▲
Plasma beta-hidroxybutyrate (μM)	11.4±1.0	654±44 ▲	19.1±2.6 O	615±138
Plasma urea (mM)	14.4±1.3	7.71±0.8 ▲	13.0±0.8	7.03±0.44 ▲
Plasma total amino acids (mM)	5.89±0.14	5.15±0.08 ▲	6.01±0.23	5.55±0.16 ▲
Plasma essential amino acids (mM)	1.83±0.09	1.62±0.03	1.65±0.06	1.81±0.07
Plasma non essential amino acids (mM)	4.05±0.07	3.53±0.06 ▲	4.36±0.17	3.74±0.13 ▲

gluconeogenic amino acids, as alanine, aspartate plus asparagine, serine and proline, partly compensated by increases in glycine and glutamate plus glutamine. There are also significant decreases in the concentrations of lysine, arginine, leucine plus isoleucine and tryptophan, in accordance with data found in the literature (12).

In the Remington diet animals, fasting provoked a more pronounced decrease in the concentrations of several amino acids, mainly alanine, proline and valine, reversing the trend observed in the controls in the case of glutamate plus glutamine, serine, threonine, lysine, arginine, histidine and tryptophan. In a general way, the differences versus controls are lower in the fasted state. This seems to suggest that the circulating amino acid levels show a trend towards a more uniform situation during fasting, with a much buffered influence of the diet as compared with the fed state, under which the differences between the groups are more marked.

In the fasted Remington diet rats, lysine and tryptophan concentrations are still significantly lower than those of controls, thus suggesting a functional lack of these essential amino acids. Their concentrations do not rise enough to attain the control values with fasting regardless of the increased amino acid output induced by peripheral tissue proteolysis and amino acid release (1) provoked by fasting (as can be seen i.e. in the increased branched chain amino acid concentrations).

The general conclusion from the present data is that the chronic use of the Remington's diet without any other nutritional supplementation can provoke experimental artifacts when the results are compared with those obtained with rats fed laboratory chow pellets. The differences are considerable, and can be tentatively attributed, not to a lack of protein or to low biological value of the protein in Remington's diet, but more probably to other unknown nutritional factor(s) lacking in that experimental diet, that impair the amino acid and lipid homeostasis of rat plasma.

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Resumen

Se compara la composición plasmática de ratas alimentadas con dieta Remington pobre en yodo (suplementada con yodo) con la de ratas alimentadas con dieta normal de laboratorio. Se observan diferencias significativas en la concentración plasmática de glicerol, aceto-acetato, β-hidroxibutirato y determinados aminoácidos, principalmente lisina y triptófano. Un ayuno de 24 horas disminuye las diferencias entre ambos grupos, aunque se mantiene la baja concentración plasmática de lisina y triptófano en las ratas alimentadas con la dieta Remington, que provoca una disminución funcional en la concentración de estos aminoácidos, a pesar de sus elevados niveles en la dieta.

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