UNIVERSITAT DE BARCELONA
Divisió de Ciències Experimentals i Matemàtiques
Facultat de Biologia
Departament de Bioquímica i Fisiologia

Balanç energètic i nitrogenat en l'obesitat genètica i nutricional de la rata

Treball que presenta

Montserrat Esteve Ràfols

per optar al grau de Doctora en Ciències Biològiques.

Barcelona, juny de 1992

treball enviat per publicar a BRITISH JOURNAL OF NUTRITION

Dietary amino acid balances in young Wistar rats fed a cafeteria diet.

Montserrat Esteve, Immaculada Rafecas, José-Antonio Fernández-López, Xavier Remesar and Marià Alemany

Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain.

SYNOPSIS

The amino acid composition of the diet ingested by reference and cafeteria diet–fed rats has been analyzed in Wistar rats from day 30 to 60 after birth. Their body protein amino acid composition as well as the urinary and faecal losses incurred were also measured.

1. Cafeteria diet resulted in a higher proportion of amino acids extracted from the diet, in spite of the fact that this diet had a very similar amino acid composition to that of the standard reference diet.

2. The net rates of accretion into body protein were higher for cafeteria diet–fed rats in the first 15 days and higher for the reference group in the second 15 days, but the net overall accumulation of protein was higher for cafeteria-fed rats.

3. Urinary losses of amino acids were small, but higher for reference diet–fed rats.

4. Cafeteria feeding results in an essentially equal amino acid intake to that from the reference diet; this higher amino acid availability is translated into higher net protein accrual and lower nitrogen losses.

5. It is postulated that the lower protein–energy proportion of the cafeteria diet —and not its amount in absolute terms— could trigger a series of amino acid–sparing mechanisms that eventually result in even higher amino acid availability, which leads to increased net protein deposition and a wider nitrogen gap.

INTRODUCTION

Most studies on nitrogen nutrition have been focused on the actual effects of dietary amino acids and protein on growing performance and other health problems (Kielanowski, 1965). The paramount importance of nitrogen metabolism in the nutrient economy of mammals is well illustrated by their marked ability to retain and recycle amino nitrogen (Waterlow & Stephen, 1981). In the event of nutritional scarcity, absorption and reutilization (Waterlow, 1985) are enhanced, and nitrogen excretion is lowered (Fashakin & Furst, 1987). On the other hand, the dietary excess of amino nitrogen —under conditions of limited energy substrate

availability— implies the necessary oxidation of amino acids for energy (Aguilar et al., 1972), greatly increasing nitrogen excretion.

However, when dietary protein and other energy substrates (carbohydrate, lipid) are widely available, there is a generalized sparing of amino nitrogen, which is not often used for energy as massively as lipids and carbohydrate (Bray, 1987). This situation is best illustrated by the experimental model of rodent nutrition known as "cafeteria" feeding (Sclafani & Springer, 1976), in which the rat selects from a wide range of appetizing foods, where lipids predominate (Prats et al., 1989). The rats fed a cafeteria diet increase their food intake (Naim et al., 1985), heat production (Rothwell & Stock, 1988), body weight and fat reserves (Barr & McCracken, 1984). They present higher circulating amino acid levels (Rafecas et al., 1992), with lower urinary nitrogen output (Henry et al., 1987), partly due to depressed operation of the urea cycle (Barber et al., 1985), as well as a wider nitrogen gap than comparable animals fed a standard diet (Esteve et al., 1992). Several reports show that cafeteria–fed rats accrue more protein —in addition to much more fat— than control reference diet–fed animals (Esteve et al., 1992), and it has been generally assumed that this is may be due in part to the higher biological value of the protein of some components of the cafeteria diets.

This study has been developed as a strict balance analysis in order to determine the differences —if any— in net amino acid availability and protein synthesis induced by an energy–rich cafeteria diet, as well as the eventual influence of amino acid composition of diet on individual amino acid management.

MATERIALS AND METHODS

Animals and diets

The animals used in this study were female Wistar rats (bred at the University of Barcelona Animal Service from Ifa-Credo stock); they were used 30 days after birth (weaned on day 22). The rats were housed individually, either in polypropylene-bottomed cages with wood shavings as absorbent material, or in polycarbonate metabolic cages (Tecniplast Gazzada, Italy) which allowed the daily estimation of the consumption of individual food items. The cages were maintained in a light (on from 08.00 to 20.00), humidity (70-80 % relative humidity) and temperature (21-22 °C) controlled environment.

Rats from the control group were fed a commercial reference pellet diet (type A04 from Panlab, Barcelona, containing 170 g/kg protein, 587 g/kg digestible carbohydrate, and 30 g/kg lipids) and tap water *ad libitum*. All studies involving the measurement of food intake were carried out with the rats kept in metabolic cages. The rats given the cafeteria diet were presented daily with a fresh offering of biscuits spread with liver pâté, bacon, banana, chow pellets (as indicated above), tap water and whole milk complemented with 333 g/l sucrose plus 10 g/l of a mineral and vitamin supplement (Gevral, Cyanamid Ibérica) (Esteve et al., 1992). All the materials were previously weighed and presented in excess (c. 20-30 % higher than the expected consumption). Twenty-four hours later, the remaining debris was isolated, identified and weighed. The drying of food leftovers was corrected for by determining the amount of water lost in one day from food samples of known weight left in a cage with no rats. This diet is a simplified version of an earlier cafeteria diet developed and studied by us (Prats et al., 1989), scaled down by selecting only the items actually consumed in significant portions. The animals were weighed every day at the same hour (11.00 to 12.00).

Experimental setup

For each dietary group (reference– and cafeteria–fed rats) three sets (5-7 rats each) of animals were studied, being killed at 30 (0 days of dietary treatment), 45 (15 days of treatment) or 60 (30 days of treatment) days after birth. The 60–day group rats were used for daily individual diet intake analysis (metabolic cages) for the whole 30 days of the study. The other rats (45–day, and 30 day old rats) were kept individually in ordinary cages, where they were fed the same diets; their intake was not measured.

On days 30, 45 or 60, the allotted groups of rats were weighed and immediately killed by decapitation. Their corpses were again weighed (the difference being the net loss of blood and fluids) and then dissected. The content of their stomach and intestines was carefully removed and weighed. The weights recorded and used for calculations were the empty body weights. The remaining carcass was then minced and ground with a blender, sampled and stored frozen at –20 °C until processing.

Analytical procedures

The ground carcasses were sampled following a previously tested sampling protocol (Esteve et al., 1992). The samples were repeatedly minced to a smooth paste with a blender; this was used for further homogenization and analysis. The constituents of the diets given to the animals were also ground and homogenized, and then subjected to the same analytical procedures. Blood samples were also analyzed and used to correct the data obtained for the different groups accounting for the blood lost during the killing and dissecting process.

The food, rat tissue, droppings and urine samples were further homogenized with a Politron, then their proteins were hydrolyzed with 6 N HCl in sealed glass tubes under nitrogen at 105 °C for 48 hours (Allen, 1989). The hydrolisates were cleaned by ultrafiltration, neutralized, and their phenyl-hydantoines were prepared (Heinrikson & Meredith, 1984). All series of determinations included standards of known amino acid mixtures. Prior to derivatization, an internal standard of norleucine was also added to each sample. The amino acid analyses were carried out in an LKB 2150 high performance liquid chromatograph using Spherisorb ODS-2 (5 μm) columns (150x4 mm) and a gradient of ammonium acetate/acetonitrile/methanol as mobile phase at 60 °C (Heinrikson & Meredith, 1984).

The losses due to the formation of Maillard adducts (Whistler & Daniel, 1985), as well as acid decomposition, and the efficiency of the derivatization process were corrected by means of internal standards and samples of known amino acid content run with each batch of samples. The values were finally adjusted with the content of nitrogen in the sample measured with a Carlo Erba NA-1500 elemental analyzer. Since the oxidation of –SH groups was higher than expected, and the recoveries of cysteine and methionine poor, these data, together with those of tryptophan —destroyed in the acid hydrolysis procedure (Hill, 1965)— were not included in the tabulated results. References to "total" amino acids do not take these non–measured amino acids into account. Each batch of each food item used in the experiments was analyzed independently. The data given in table 1 are mean food composition values, but in all cases they were computed from the composition data of the actual food batches used. No statistically significant deviations of composition in any of the food items analyzed were found.

Calculations and statistics

The analyses of body composition of the series of rats killed on days 30 and 45 were used for the calculation of the composi-

tion of the rats kept in metabolic cages (killed at day 60, after one month of daily measurement of intake and body weight) on days 30 and 45. Since their weights were very close ($P > 0.95$, Student's *t* test) and the feeding scheme was the same, it was assumed that the percentage composition of the two series of rats was identical for matching age and diet.

The amino acid composition of the samples, and the mass of the rat carcass allowed the estimation of the amount of each amino acid present in each of the two age-groups studied, and also allowed the quantitative estimation of the differences between the amino acids stored, always related to the actual empty body weight of the animal and referred to the age of comparison.

The known amount of each food item consumed daily by each rat and its amino acid composition (Table 1) were used to establish the amount of each amino acid ingested by each rat for each day. The tabulated data for each diet component were combined in order to determine the gross intake of each amino acid during each of the 15-day periods studied. Statistical comparisons between the groups were made with a standard two– or three–way ANOVA program, complemented with the Tuckey test, or by using the Student's *t* test, as well as the χ^2 test.

RESULTS

The amino acid composition of the food ingested by the rats fed the cafeteria diet was practically identical to that of the protein contained in the reference diet (Figure 1). The amount of amino acid ingested by rats fed the reference diet was 305.1 ± 9.6 mmoles (mean ± SEM) during the first 15 days of the experiment, and 332.1 ± 6.4 in the second; cafeteria–fed rats ingested, respectively, 306.3 ± 8.1 and 323.3 ± 9.3 mmoles. The amount of energy ingested by reference diet fed rats was 7.13 ± 0.09 MJ in the 30-day period, significantly less than the 8.87 ± 0.14 MJ ingested by cafeteria-fed rats. The protein-derived energy ingested was, then, a mean 21.7 % for reference diet and 14.3 % for cafeteria-fed rats.

The individual amino acids ingested and not absorbed (i.e. those present in the droppings) for the whole 30-day period are presented in Figure 2. Cafeteria-fed rats lose less amino nitrogen —absorbed a larger proportion— in the faeces than their reference diet–fed counterparts, a mean 12.7 ± 0.7 % for the cafeteria diet and 19.1 ± 1.3 % for the reference. The differences were also statistically significant for some individual amino acids (Ala, Ser, Hyp, Tyr, Thr and Lys). The proportion of each amino acid not absorbed and lost was not uniform. The amino acid lost in largest proportion was Ala, and those absorbed in the highest percentage were Glu + Gln (Glx).

The total amino acid content at 30, 45 and 60 days of rats fed the reference or cafeteria diet are shown in Figure 3. The amount of each amino acid present in the body of the rat increased significantly with time. In general, rats fed the cafeteria diet accrued more amino acid than those fed the reference diet, the differences being significant for Glx, Ala, Ser, Tyr, Phe, Val, Ile, Thr and His. The maximal differences were found in 45 day old rats.

The net rates of amino acid incorporation into the body stores (essentially protein) are shown in Table 2. When the data presented in the table are expressed as a proportion of the whole individual amino acid mass of the rat, the rates of accrual become considerably uniform, with figures in the range of the data given in the table for total protein. The decrease observed for rats fed the reference diet from days 30-45 to 45-60 were considerable (a mean drop of about 10

Table 1

Amino acid and energy content of the foods offered to cafeteria-fed rats

amino acid	chow pellet	liver pâté	bacon	banana	biscuits	milk
Glx (mmol/kg)	272 ± 20	136 ± 9	184 ± 7	9.4 ± 0.7	98 ± 5	43 ± 5
Asx (mmol/kg)	116 ± 9	84 ± 7	130 ± 5	10.9 ± 0.7	12 ± 1	18 ± 2
Ala (mmol/kg)	60 ± 5	46 ± 5	82 ± 3	5.4 ± 0.7	14 ± 1	8 ± 1
Ser (mmol/kg)	77 ± 7	50 ± 4	71 ± 3	6.3 ± 0.4	19 ± 1	15 ± 2
Gly (mmol/kg)	121 ± 9	100 ± 11	184 ± 10	9.6 ± 0.9	25 ± 1	8 ± 1
Pro (mmol/kg)	138 ± 5	67 ± 5	94 ± 4	5.6 ± 0.6	56 ± 4	26 ± 2
Hyp (mmol/kg)	5 ± 1	15 ± 11	43 ± 3	1.0 ± 0.1	0 ± 0	0 ± 0
Arg (mmol/kg)	55 ± 4	40 ± 4	75 ± 3	5.0 ± 0.3	7 ± 1	6 ± 1
Tyr (mmol/kg)	12 ± 1	18 ± 1	24 ± 1	1.6 ± 0.2	2 ± 0	4 ± 1
Phe (mmol/kg)	46 ± 3	32 ± 2	41 ± 1	3.7 ± 0.3	13 ± 1	8 ± 1
Val (mmol/kg)	68 ± 7	58 ± 4	77 ± 3	5.6 ± 0.4	14 ± 1	15 ± 2
Leu (mmol/kg)	90 ± 7	69 ± 4	92 ± 4	7.6 ± 0.4	24 ± 0	20 ± 3
Ile (mmol/kg)	55 ± 4	43 ± 3	68 ± 2	4.7 ± 0.1	13 ± 1	12 ± 2
Thr (mmol/kg)	51 ± 4	42 ± 4	85 ± 2	6.4 ± 0.9	12 ± 1	11 ± 1
Lys (mmol/kg)	49 ± 4	55 ± 5	97 ± 9	4.7 ± 0.4	4 ± 0	12 ± 1
His (mmol/kg)	43 ± 4	15 ± 1	31 ± 2	7.2 ± 0.9	8 ± 1	7 ± 1
EAA/NEAA ratio[1]	0.47	0.56	0.53	0.73	0.38	0.65
protein (g/kg)	170 ± 7.2	123 ± 13	173 ± 5	14 ± 1	63 ± 1	22 ± 2
carbohydrate (g/kg)	587 ± 20	7.0 ± 2.1	0.0 ± 0.0	171 ± 12	655 ± 34	319 ± 14
lipid (g/kg)	30 ± 1	291 ± 22	299 ± 43	1.0 ± 0.1	155 ± 3	19 ± 3
energy (MJ/g)	14.6	13.5	14.6	3.3	18.5	6.4
protein energy (%)	21.7	17.0	22.1	7.9	6.3	6.4

[1] Essential amino acids [Phe + Val + Leu + Ile + Thr + Lys + His] versus non-essential amino acids [Glx + Asx + Ala + Ser + Gly + Pro + Hyp + Arg + Tyr] ratio

% in the mean daily rate of accretion, which represents a decrease of about 51 % in this rate expressed as a percent of the rat mean net daily change in protein stores. The figures for cafeteria-fed rats were initially higher (30-45 days) than those of rats fed the reference diet, but the decrease observed in the second part of the study was much more steep: mean decreases of about 52 % in daily rates of amino acid incorporation, which were translated into a 74 % decrease in these rates referred to total protein.

When comparing the 30-45 and 45-60 day periods, individual amino acids showed a different pattern of change. The rats fed the reference diet presented more pronounced decreases for Ser, Hyp, Val, Thr and Lys net incorporation, with small or nil change in Glu + Gln, Ala, Phe, Leu and His, and increases in Asp + Asn, Gly and Pro. The rats given the cafeteria diet presented steep and uniform decreases, maximal for Ala, Ser, Hyp, Tyr and Thr, and less pronounced for Glu + Gln, Asp + Asn and Gly. These different changes suggest a slight shift in overall body composition in both sets of rats as a function of changing age.

In Figure 4 the fate of dietary amino acids is summarized in a stacked–column diagram. The overall effect of diet on the distribution of these amino acids was statistically significant for all fractions. The measurement of intake, accumulation and losses allowed the calculation of the amount of each amino acid theoretically available for metabolic utilization other than net protein synthesis. The net deposition of hydroxyproline, added to the losses through urine and droppings was in excess of the amount ingested in either diet; the situation for alanine in the reference diet was very similar, *being deposited in excess of the amount available* to the rat. In general, there was a larger proportion of each individual amino acid available for oxidation or metabolic use in cafeteria diet-fed rats, whereas the rats fed the reference diet lost or accrued some amino acids in proportions close to those ingested (Ala, Gly, Tyr and Lys).

The urinary excretion of individual amino acids for reference and cafeteria diets is presented in Table 3. Most amino acids were excreted in very small proportion except for Hyp, Gly and Ala. The dietary treatment resulted in different proportions of amino acids lost in

Table 2

Mean daily rates of amino acid incorporation into body protein

amino acid	reference diet 30-45 days	reference diet 45-60 days	cafeteria diet 30-45 days	cafeteria diet 45-60 days
Glx	1027	1002	1091	977
Asx	653	718	675	640
Ala	610	613	832	255
Ser	563	343	806	105
Gly	1125	1445	1139	927
Pro	633	694	699	316
Hyp	381	161	392	24
Arg	404	294	473	144
Tyr	142	108	201	43
Phe	213	211	265	144
Val	427	227	479	171
Leu	616	632	759	350
Ile	313	250	378	169
Thr	451	269	630	41
Lys	714	467	788	271
His	119	111	217	115
total [1]	8.40	7.55	9.83	4.70
total [2]	4.76	2.34	5.15	1.33

The data are expressed in μmoles of amino acid residues incorporated into the rat protein per day.
[1] expressed in mmoles of amino acid residues incorporated per day
[2] expressed as a percentage of the rat protein pool.

Table 3

Urinary amino acid losses of rats fed reference and cafeteria diets

amino acid	reference diet 30-45 days	reference diet 45-60 days	cafeteria diet 30-45 days	cafeteria diet 45-60 days	p values (ANOVA) diet	control cafeteria time	cafeteria time
Glx	0.18 ± 0.02	0.22 ± 0.03	0.20 ± 0.02	0.21 ± 0.02	0.9442	0.2836	0.7669
Asx	0.48 ± 0.06	0.51 ± 0.11	0.34 ± 0.03	0.35 ± 0.03	0.0396	0.7860	0.9594
Ala	2.85 ± 0.54	7.87 ± 2.95	1.17 ± 0.10	1.38 ± 0.08	0.0133	0.0283	0.9204
Ser	0.39 ± 0.05	0.43 ± 0.08	0.36 ± 0.05	0.35 ± 0.08	0.4305	0.7170	0.9174
Gly	3.40 ± 0.38	3.90 ± 0.89	1.63 ± 0.10	2.11 ± 0.23	0.0019	0.4876	0.5049
Pro	0.18 ± 0.04	0.13 ± 0.02	0.08 ± 0.01	0.06 ± 0.01	0.0011	0.1682	0.4363
Hyp	11.86 ± 2.81	0 ± 0	7.66 ± 1.49	3.56 ± 0.71	0.8461	0.0001	0.0916
Arg	0.14 ± 0.02	0.15 ± 0.04	0.19 ± 0.03	0.20 ± 0.02	0.0892	0.8354	0.8354
Tyr	0 ± 0	0 ± 0	0 ± 0	0 ± 0	—	—	—
Phe	0.97 ± 0.10	0.18 ± 0.06	0.14 ± 0.02	0.27 ± 0.04	0.0000	0.0000	0.1476
Val	0.09 ± 0.01	0.04 ± 0.01	0.10 ± 0.01	0.05 ± 0.01	0.3963	0.0048	0.0048
Leu	0 ± 0	0 ± 0	0.04 ± 0.00	0 ± 0	0.0000	1.0000	0.0000
Ile	0 ± 0	0 ± 0	0 ± 0	0 ± 0	—	—	—
Thr	0.67 ± 0.05	0.76 ± 0.12	0.41 ± 0.06	0.65 ± 0.07	0.0346	0.04001	0.0502
Lys	0.44 ± 0.06	0.78 ± 0.08	0.37 ± 0.06	0.26 ± 0.04	0.0001	0.0010	0.2487
His	0.33 ± 0.03	0.44 ± 0.04	0.70 ± 0.11	0.81 ± 0.08	0.0000	0.3182	0.2606

The data are expressed as percentage of absorbed (ingested minus lost in the faeces) amino acids.

the urine; in general they were higher for reference diet–fed rats than for those given the cafeteria diet, with the exception of His, and Hyp as well as Phe for the second period of time studied. The quantity of some amino acids excreted through the urine

Table 4

Minimum (balance) amino acids metabolized by rats fed a reference or cafeteria diet

amino acid	reference diet 30-45 days	reference diet 45-60 days	cafeteria diet 30-45 days	cafeteria diet 45-60 days	p values (ANOVA) diet	control time	cafeteria time
Glx	73.7 ± 0.5	76.2 ± 0.45	68.1 ± 1.4	73.5 ± 1.7	0.0035	0.1956	0.0069
Asx	56.6 ± 1.1	56.3 ± 2.7	57.4 ± 1.5	62.0 ± 2.2	0.1181	0.9210	0.1162
Ala	−15.2 ± 5.8	−15.7 ± 13.9	−18.3 ± 3.6	64.8 ± 4.7	0.0001	0.9655	0.0000
Ser	43.0 ± 1.3	67.9 ± 2.8	22.6 ± 2.9	90.7 ± 2.1	0.6180	0.0000	0.0000
Gly	19.4 ± 3.0	4.5 ± 6.4	25.1 ± 2.7	37.0 ± 5.2	0.0005	0.0321	0.3948
Pro	67.3 ± 0.8	67.2 ± 2.0	60.2 ± 2.1	84.1 ± 1.7	0.0094	0.9892	0.0000
Hyp	−584.6 ± 55.3	−139.2 ± 31.6	−145.9 ± 17.0	82.9 ± 7.8	0.0000	0.0000	0.0001
Arg	47.9 ± 0.7	65.4 ± 2.5	38.1 ± 1.8	81.1 ± 2.4	0.1560	0.0000	0.0000
Tyr	3.24 ± 1.8	26.0 ± 6.0	14.7 ± 1.8	83.4 ± 2.4	0.0000	0.0002	0.0000
Phe	63.3 ± 1.2	67.0 ± 2.1	53.6 ± 1.6	76.4 ± 1.9	0.9401	0.1563	0.0000
Val	49.7 ± 0.8	75.4 ± 2.7	51.2 ± 1.7	84.7 ± 1.7	0.0085	0.0000	0.0000
Leu	46.4 ± 1.7	49.7 ± 3.4	41.4 ± 1.9	76.1 ± 2.0	0.0002	0.3376	0.0000
Ile	57.6 ± 0.5	69.1 ± 2.2	53.0 ± 1,5	81.6 ± 1.6	0.0217	0.0001	0.0001
Thr	23.4 ± 3.5	57.4 ± 3.7	13.2 ± 1.7	94.4 ± 2.1	0.0002	0.0000	0.0000
Lys	2.3 ± 3.9	23.4 ± 6.2	7.2 ± 3.0	71.8 ± 2.7	0.0000	0.0000	0.0000
His	74.4 ± 0.7	81.7 ± 1.8	57.9 ± 1.9	78.9 ± 2.0	0.0000	0.0064	0.0000

The data are expressed as percentage of absorbed (ingested minus lost in the faeces) amino acids.

changed dramatically with time, especially for reference diet-fed rats: increases in Ala and Lys excretion and decreases in Phe, and —especially— Hyp. For cafeteria-fed rats the changes with time were less marked. The only differences were an increase in the urinary rates of excretion of Phe and a decrease of Hyp in the second 15-day period compared with the first.

Table 4 presents the balance of amino acids absorbed not excreted and not accrued, i.e. the minimum proportion of each amino acid necessarily metabolized. In most cases, the amount of each amino acid absorbed but not accrued was higher for rats fed the cafeteria diet, this effect being practically universal for the 45-60 day period; in the first 15-day stretch, this trend was already observed for Gly, Tyr, Lys and Hyp, whereas the metabolic utilization of Ser, Thr and Arg was higher in reference diet-fed rats. There were marked changes in the amount of each amino acid metabolized in either period for each diet. In general, the amount of amino acids absorbed but not accrued in the second 15-day period was higher than in the first, the trend being more marked for cafeteria-fed rats. The only exceptions were Gly and Hyp, the latter reflecting only a decrease in deposition.

DISCUSSION

The precise formulation of most standard diets used for growth and maintenance of the rat as a laboratory animal has been well established (Rogers, 1979), at least in reference to the amount and quality of the protein (Miller, 1969). On the other hand, it has often been claimed that the self-selected diets are less uniform in terms of energy, amino acid and microcomponent content (Moore, 1987). The rat has a tight control of its energy and nutrient intake (Oscai & McGarr, 1978), that drives the animal to eat varying amounts of food in order to obtain the energy and protein nitrogen (Cohn & Joseph, 1962) it needs for maintenance and growth. Despite some of its usual components containig high biological quality protein, some reports suggested that the cafeteria diets could provide insufficient protein (Sclafani & Springer, 1976), but this aspect has not been studied in depth. Rats offered a cafeteria diet select a fairly constant amount of dietary protein, although they may ingest a much greater share of other energy components (Anderson, 1979), essentially fats (Barber et al., 1987). In this experiment we have observed that the protein actually selected by the rats fed the cafeteria diet showed an amino acid composition essentially indistinguishable from that present in the standard reference diet used. This situation was accomplished by each rat selecting different —and rather variable— morsels of the different food items available every day, in spite of the markedly dissimilar amino acid composition of the different foods offered. The similarity between the reference and cafeteria diets amino acid composition is not limited to the essential amino acids, since it extends to the non-essential. All these data give weight to the notion that both diets used were equal and comparable in amino acid composition and —thus— in biological quality. In this way, we can compare the fate of each individual amino acid from an intake at two different levels of energy but with a uniform protein fraction, with the additional advantage that in either case the rats are fed *ad libitum*, with no additional dietary constrictions or manipulation.

A remarkable difference in amino acid handling by the rats fed the reference or cafeteria diets was the higher dietary protein digestion / absorption efficiency of the former. This resulted in increased availability of dietary amino acids, and may help explain their high circulating levels (Calles-Escandon et al., 1984), as well as the increased rates of growth (Rothwell & Stock, 1979) and nitrogen deposition (Barber et al., 1985) induced by the cafeteria diets. Since the rates of absorption for the different amino acids were neither constant nor uniform, the variability may be a consequence of diet-induced changes in the intestinal amino acid transport systems. This is in agreement with the modulation of amino acid transport in the rat intestine by energy availability (Karasov et al., 1987), or diabetes (Schedl, 1974) and other alterations (Israel et al., 1968). On the other hand, amino acid availability influences the effectiveness of its transport into the rat tissues (Casado et al., 1987).

In the rat, cafeteria feeding results in higher body weights (Rothwell & Stock, 1979) and increased fat deposition (Barr & McCracken, 1984); the effects being more marked when the diet is offered during and after weaning (Salvadó et al., 1986). This increase is accompanied by parallel —but less extensive— increases in lean body mass (Maxwell et al., 1988), with enhanced protein deposition in absolute terms (Barr & McCracken, 1984). This is in part driven by a higher amino nitrogen availability, paralleled by lowered urea nitrogen excretion (Barber et al., 1985). However, the nitrogen losses of cafeteria-fed rats may be in a similar range to that of those fed the reference diet, since cafeteria diets may generate

a higher nitrogen gap (i.e. nitrogen ingested but not found in the excreta nor accrued in the body of the experimental animals) (Esteve et al., 1992).

The data presented here suggest that the rates of protein accretion are markedly influenced by the diet, but even more by the age —or perhaps size— of the animals: cafeteria-fed rats grew faster, up to higher body protein settings; but later on, their net protein deposition rates slowed more steeply than in rats fed the reference diet, which maintained a more uniform rate throughout the 30 days studied. The tapering off net protein–deposition rates is evident even when it is expressed as a percent of the total protein mass of the rat. It cannot be attributed to lowered amino acid levels (Rafecas et al., 1992), nor to age–decreased amino acid availability, since the proportion of all amino acids absorbed —and thus available for protein deposition or other metabolic uses— was higher in cafeteria–fed rats.

The very low net oxidation of lysine suggests that it can be a limiting amino acid for both diets in the —fast-growth— 30-45 day period.

The analysis of urinary amino acid losses did not shed any additional light on this puzzle, since they were small and in the same range —but lower for cafeteria rats— for both sets of animals in either 15-day period. Here again, the differences could be attributed to altered renal clearance or reabsorption of most amino acids. In any case, the very small proportion of amino acids lost as such in the urine could not have a significant effect on the amino acid balance (Pastor-Anglada & Remesar, 1986).

Rats fed a cafeteria diet excrete less urinary and fecal (Henry et al., 1987) nitrogen than the animals fed standard diets; however, their nitrogen balances present much wider nitrogen gaps (Esteve et al., 1992). The data observed here may give support to the existence of such a gap when the slowing in accretion rates for the 45-60 day period was combined with the high net dietary amino acid availability, the intestinal absorption was higher than for the reference diet. This nitrogen is not excreted through the urine, which agree with earlier data in which only total nitrogen balance was measured (Barber et al., 1985; Esteve et al., 1992).

The mean protein amino acid composition of the rat showed only small changes with age or diet, but some amino acids (i.e. Ala, Gly and Pro) were incorporated in a somewhat larger proportion into the protein of reference diet–fed rats, which also showed a higher presence of these amino acids on day 60. This may be due to the higher relative surface area with respect to volume of the leaner reference diet–fed rats in comparison with the fatter cafeteria diet–fed ones. This higher area could mean a higher mass of skin (and thence of collagen) for the reference diet–fed animals, thus partly explaining the higher presence of amino acids well represented in collagen (Bornstein & Traub, 1979).

The most striking difference between the two diets as to amino acid management is found in the changes induced in amino acid metabolism by diet composition: not by protein availability or quality —essentially identical for both diets tested—, but by the other components of the diet or the ratio of protein content versus other energy substrates. The presence of fiber in the reference diet (4.3 % of dry weight) is not too far from the calculated fiber content in the mean cafeteria diet ingested by the rats (3.1 % of dry weight), but it can have at least some effect in the duration of the digestion/absorption process (Farrell & Arthur, 1978). The small difference in proportion is even lower if we calculate the amount of fiber actually ingested (18.5 ± 0.4 g and 14.3 ± 0.6 g in

30 days for, respectively, reference and cafeteria diet), thus making it more difficult to attribute the effects observed in amino acid handling to the small differences in the diet fiber content.

Since the difference in the levels of energy derivable from protein in the diet ingested was small (1.55 ± 0.02 MJ and 1.27 ± 0.02 MJ, respectively for reference and cafeteria diets in 30 days, i.e. 21.7 % and 14.3 % of the ingested energy), as it was for carbohydrate (5.01 ± 0.07 MJ and 5.26 ± 0.02 MJ, respectively for reference and cafeteria diets in 30 days, i.e. 70.3 % and 59.3 % of the ingested energy), but not for the lipid fraction (0.56 ± 0.01 MJ and 2.44 ± 0.11 MJ, respectively for reference and cafeteria diets in 30 days, i.e. 7.9 % and 27.5 % of the ingested energy), it can be postulated that it is the excess (lipid) energy the element that propels all changes observed in amino acid metabolism by the cafeteria-diet.

The excess energy intake did not actually affect the appetite for protein nor its ingestion, which has been postulated to be set by an independent mechanism than energy intake (Ashley & Anderson, 1977). Then, the rats fed cafeteria diet ate essentially the same protein as did those fed on the reference diet, but they absorbed, accrued and metabolized these amino acids very differently. In general, the amino acids were more available and thus accrued in a larger proportion in rats fed the cafeteria diet, i.e. they saved their amino acids more efficiently from a diet with a lower proportion of protein energy in its composition. It can be postulated that the lower relative presence of protein energy —not amount— of the cafeteria diet may induce the same protein-sparing mechanisms observed in situations of amino acid scarcity: starvation (Cahill, 1970) or diluted diets (Booth, 1974). In all these cases, there is a lower amino acid oxidation with decreased urea production (Sapir & Walser, 1977), but circulating amino acids are maintained within acceptable limits by limited proteolysis (Hoffer & Forse). There is a conflict between the setting of amino acid sparing mechanisms —lower urea production, higher intestinal absorption, decreased urinary N excretion, etc. (Waterlow, 1986)— and the higher availability derived form the ingestion of roughly the same amount of amino acids and the effectiveness of the saving mechanisms. Thus, the only outlets available for these amino acids are higher accrual and the unexplained and not understood mechanisms that are defined as nitrogen gap (Esteve et al., 1992), and have been postulated as production of nitrogen gas (Costa et al., 1968; Cissik et al., 1972).

ACKNOWLEDGEMENTS

Work supported by a grant PB88-0208 from the 'Dirección General de Investigación Científica y Técnica' from the Government of Spain. The authors acknowledge the technical assistance of Maria Reixach, Dr. Isidre Canals and Pilar Fernández from the Scientific and Technical Services of the University of Barcelona for their help in obtaining the aminograms. Thanks are given to Robin Rycroft for his help in the edition of the manuscript.

REFERENCES

Aguilar, T.S., Harper, A.G. & Benevenga, N.J. (1972) Efficiency of utilization of indispensable amino acids for growth by the rat. *J. Nutr.* **102,** 1199-1208.

Allen, G. (1989) Sequencing of protein and peptides. In: *Laboratory Techniques in Biochemistry and Molecular Biology*, pp.40-42, vol. 9 [R.H. Burdon & P.M. van Knippenberg, editors]. Amsterdam: Elsevier.

Anderson, G.H. (1979) Control of protein and energy intake: role of plasma amino acids and brain neurotransmitters. *Can. J. Physiol. Pharmacol.* **57,** 1043-1057.

Ashley, D.V.M. & Anderson, G.H. (1977) Protein intake regulation in the weanling rats: effect of additions of lysine, arginine and ammonia on the selection of gluten and energy. *Life Sci.* **21,** 1235-1244.

Barber, T., Estornell, E., Estelles, R., Gómez, D. & Cabo, J. (1987) Studies on the role of insulin in N metabolism changes in cafeteria–fed rats. *Mol. Cell. Endocrinol.* **50,** 15-22.

Barber, T., Viña, J.R., Viña, J. & Cabo, J. (1985) Decreased urea synthesis in cafeteria–diet induced obesity in rat. *Biochem. J.* **230,** 675-681.

Barr, H.G. & McCracken, K.J. (1984) High efficiency of energy utilization in "cafeteria", and force fed–rats left at 29 °C. *Br. J. Nutr.* **51,** 379-387.

Booth, D.A. (1974) Food intake composition for increase or decrease in the protein content of the diet. *Behav. Biol.* **12,** 31-40.

Bornstein, P. & Traub, W. (1979) The chemistry and biology of collagen. In: *The Proteins*, pp. 411-632 [H. Neurath & R.L. Hill]. New York, Academic Press.

Bray, G.A. (1987) Obesity– A disease of nutrient or energy balance?. *Nutr. Rev.* **45,** 33-47.

Cahill, G.F. (1970) Starvation in man. *New Engl. J. Med.* **282,** 668-675.

Calles-Escandon, J., Cunningham, J. & Felig, P. (1984) The plasma amino acid response to cafeteria feeding i the rat: influence of hyperphagia, sucrose intake, and exercice. *Metabolism* **33,** 364-368.

Casado, J., Remesar, X. & Pastor-Anglada, M. (1987) Hepatic uptake of amino acids in late-pregnant rats. Effect of food deprivation. *Biochem. J.* **248,** 117-122.

Cissik, J.H., Johnston, R.E. & Rokosch, D.K. (1972) Production of gaseous nitrogen in human steady-state conditions. *J. Appl. Physiol.* **32,** 155-159.

Cohn, C. & Joseph, D. (1962) Influence of body weight and body fat on appetite of normal lean and obese rats. *Yale J. Biol. Med.* **34,** 598-601.

Costa, G., Ullrich, L., Kantor, F. & Holland, J.F. (1968) Production of elemental nitrogen by certain mammals including man. *Nature* **218,** 546-551.

Esteve, M., Rafecas, I., Remesar, X. & Alemany, M. (1992) Nitrogen balances of lean and obese Zucker rats subjected to a cafeteria diet. *Int. J. Obesity* **16,** in the press.

Farrell, D.J., Girle, L. & Arthur, J. (1978) Effects of dietary fibre on the apparent digestibility of major food components and on blood lipids in men. *Aust. J. Exp. Biol. Med. Sci.* **56,** 469-479.

Foshakin, J.B. & Furst, P. (1987) Effect of dietary protein restriction on urinary nitrogen loss and tissue free amino acid patterns in mature rats. *Nutr. Res.* **7,** 285-298

Heinrikson, R.L. & Meredith, S.C. (1984) Amino acid analysis by reverse–phase high–performance liquid chromatography: Precolumn derivatization with phenylisothiocyanate. *Anal. Biochem.* **136,** 65-74.

Henry, C.J.R., Rivers, J.P.W. & Rayne, P.R. (1987) Reduced obligatory nitrogen loss in rats made obese by cafeteria feeding. *Nutr. Res.* **7,** 1243-1252.

Hill, R.L. (1965) Hydrolysis of proteins. In: *Advances in Protein Chemistry*, pp. 37-107, vol. 20 [C.B. Anfnsen, M.L. Anson, J.T. Edsall & F.M. Richards, editors]. New York: Academic Press.

Hoffer, L.J. & Forse, R.A. (1990) Protein metabolic effects of a prolonged fast and hypocaloric refeeding. *Am. J. Physiol.* **258,** E832-E840.

Israel, Y., Salazar, I. & Rosenmann, E. (1968) Inhibitory effects of alcohol on intestinal amino acid transport in vivo and in vitro. *J. Nutr.* **96,** 499-504.

Karasov, W.H., Solberg, D.H. & Diamond, J.M. (1987) Dependence of intestinal amino acid uptake on dietary protein on amino acid levels. *Am. J. Physiol.* **252,** G614-G625.

Kielanowski, J. (1965) Estimates of the energy cost of protein deposition in growing animals. In: *Energy metabolism*, pp.13-19 [K.L. Blaxter, editor]. London: Academic Press.

Maxwell, G.M., Fourie, F. & Bates, D.J. (1988) The effect of unrestricted "cafeteria" diets upon the energy exchange and body composition of weanling rats. *Nutr. Rep. Int.* **37,** 629-637.

Miller, S.A. (1969) Protein metabolism during growth and development. In: *Mammalian Protein Metabolism*, pp. 183-227 [H.N. Munro & J.B. Allison, editors]. New York: Academic Press.

Moore, B. (1987) The cafeteria diet —An inappropriate tool for studies of thermogenesis. *J. Nutr.* **117,** 227-231.

Naim, M., Brand, J., Kare, M.R. & Carpenter, R.G. (1985) Energy intake, weight gain and fat deposition in rats fed flavored, nutritionally controlled diets in a multichoice "cafeteria" design. *J. Nutr.* **115,** 1447-1458.

Oscai, L.B. & McGarr, J.A. (1978) Evidence that the amount of food consumed in early life fixes appetite in the rat. *Am. J. Physiol.* **235,** R141-R144.

Pastor-Anglada, M. & Remesar, X. (1986) Urinary amino–acid excretion in the pregnant rat. *Nutr. Res.* **6,** 709-718.

Prats, E., Monfar, M., Castellà, J., Iglesias, R. & Alemany, M. (1989) Energy intake of rats fed a cafeteria diet. *Physiol. Behav.* **45,** 263-272.

Rafecas, I., Esteve, M., Remesar, X. & Alemany, M. (1992) Plasma amino acids of lean and obese

Zucker rats subjected to a cafeteria diet. *Biochem. Internat.* in the press.

Rogers, A.E. (1979) Nutrition. In: *The Laboratory Rat*, pp. 123-152, vol. 1 [H.J. Baker, J.R. Lindsey & S.H. Weisbroth, editors]. New York: Academic Press.

Rothwell, N.J. & Stock, M.J. (1988) The cafeteria diet as a tool for studies of thermogenesis. *J. Nutr.* **118,** 925-928.

Rothwell, N.J. & Stock, M.J. (1979) A role for brown adipose tissue in diet–induced thermogenesis. *Nature* **281,** 31-35.

Salvadó, J., Segués, T., Alemany, M. & Arola, Ll. (1986) Effects of lactation on circulating plasma metabolites in "cafeteria-fed" rats. *Br. J. Nutr.* **55,** 139-147.

Sapir, D.G. & Walser, M. (1977) Nitrogen sparing induced early in starvation by infusion of branched-chain ketoacids. *Metabolism* **26,** 301-308.

Schedl, H.P. (1974) Intestinal adaptation in diabetes: amino acid and carbohydrate absorption. In: *Intestinal Adaptation*, pp. 205-216 [R.H. Dowling & O.R. Riecken, editors]. Stuttgart: Schattanes Verlag.

Sclafani, A.A. & Springer, D. (1976) Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. *Physiol. Behav.* **17,** 461-471.

Waterlow, J.C. (1985) What do we mean by adaptation?. In: *Nutrition adaptation in man*, pp.1.10 [K.L. Blaxter, J.C. Waterlow, editors]. London: John Libbey.

Waterlow, J.C. (1986) Metabolic adaptations to low intakes of energy and protein. *Annu. Rev. Nutr.* **6,** 495-526.

Waterlow, J.C. & Stephen, J.M.L. (1981) Nitrogen metabolism in man. *Biochem. J.* **73,** 277-286.

Whistler, R.L. & Daniel, J.R. (1985) Carbohydrates. In: *Food Chemistry*, pp.69-137 [O.R. Fennema, editor] New York: Marcel Dekker.

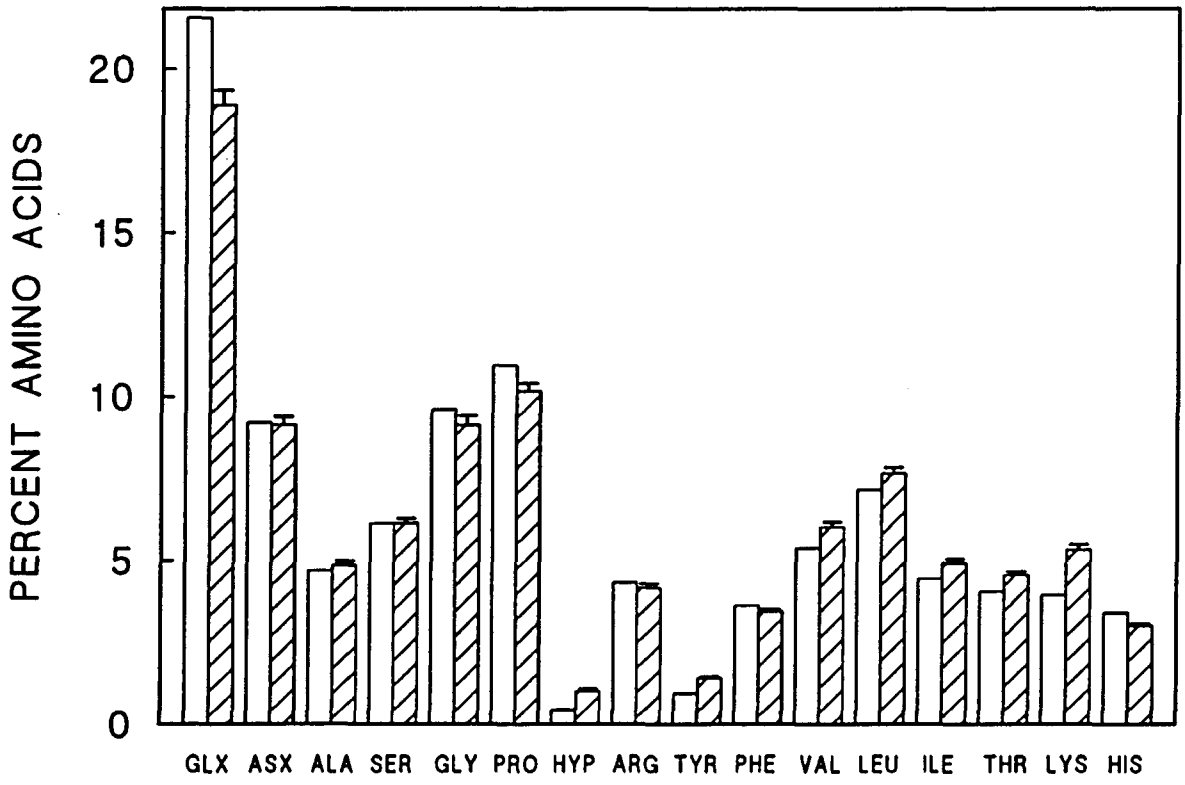
LEGENDS TO FIGURES

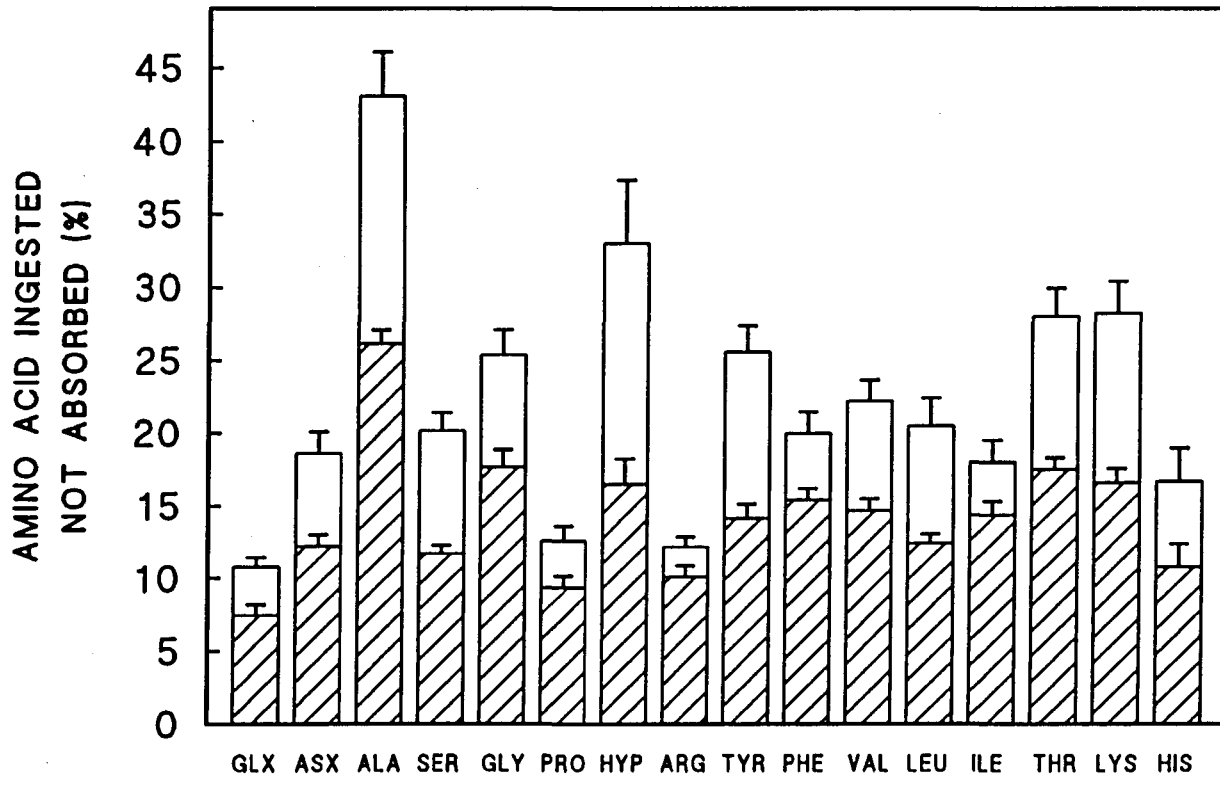
Figure 1 — AMINO ACID COMPOSITION OF THE DIETS ACTUALLY INGESTED BY THE RATS FED A REFERENCE OR A CAFETERIA DIET.
White bars correspond to the reference diet; dashed bars correspond to the cafeteria diet (i.e. the mean composition of the food ingested by cafeteria-fed rats).
Statistical comparison between diets (χ^2 test), i.e. probability of both sets of data being equal: P=1.0000 (df=15).

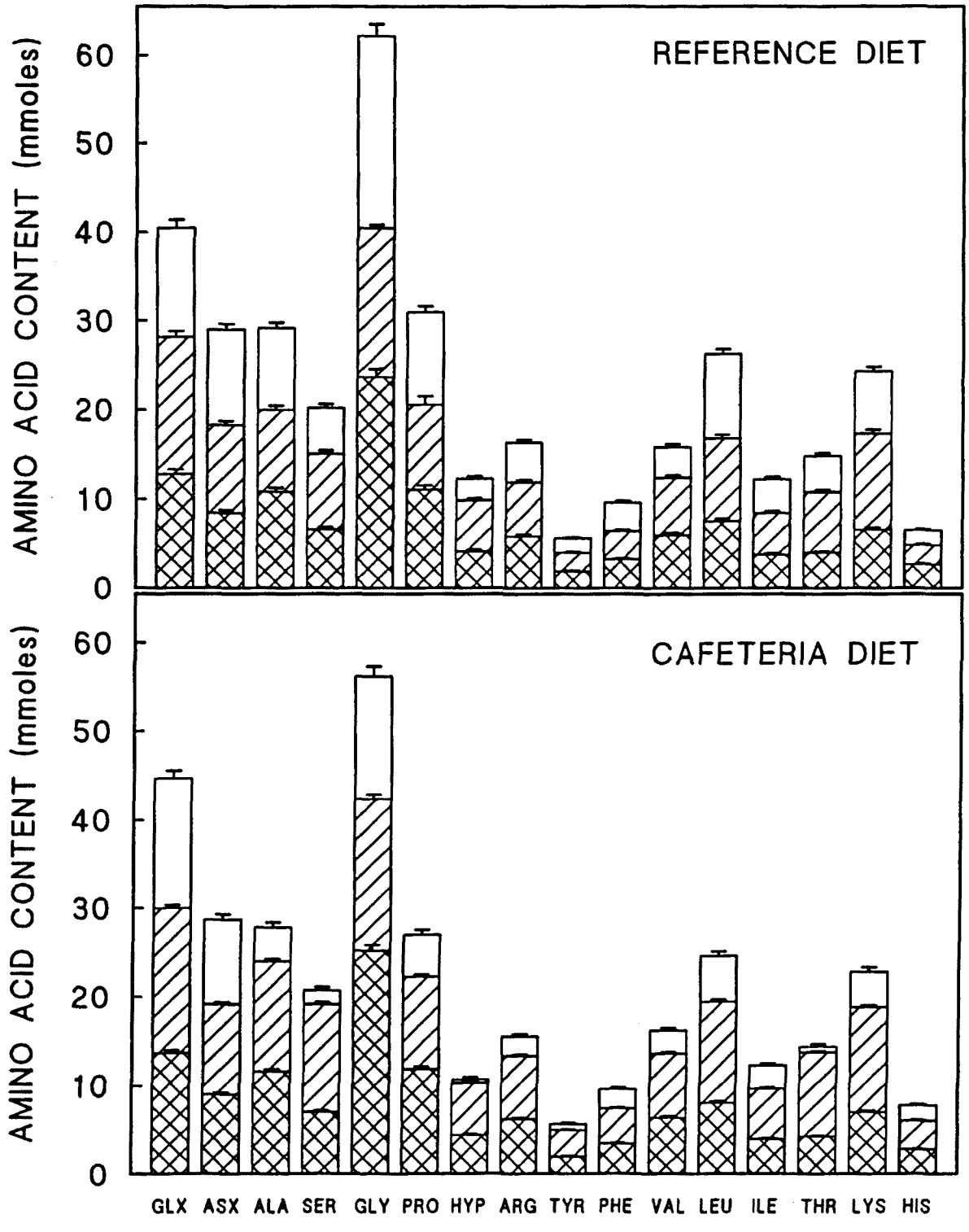
Figure 2 — PERCENT OF AMINO ACIDS INGESTED IN THE DIET BUT NOT ABSORBED.
White (higher) columns represent the reference diet, and the dashed columns the cafeteria diet. All data refer to the 30-60 days period, and are presented as mean ± SEM of six different animals per group.
*Statistical comparison of the data (two-way ANOVA): The effect of **diet** is significant (P=0.0000), as are the differences between **amino acids** (i.e. the percentage of amino acids not absorbed is not uniform) (P=0.0000). The effect of diet on individual amino acids is significant (P<0.05; Tuckey test) for Ala, Ser, Hyp, Tyr, Thr and Lys.*

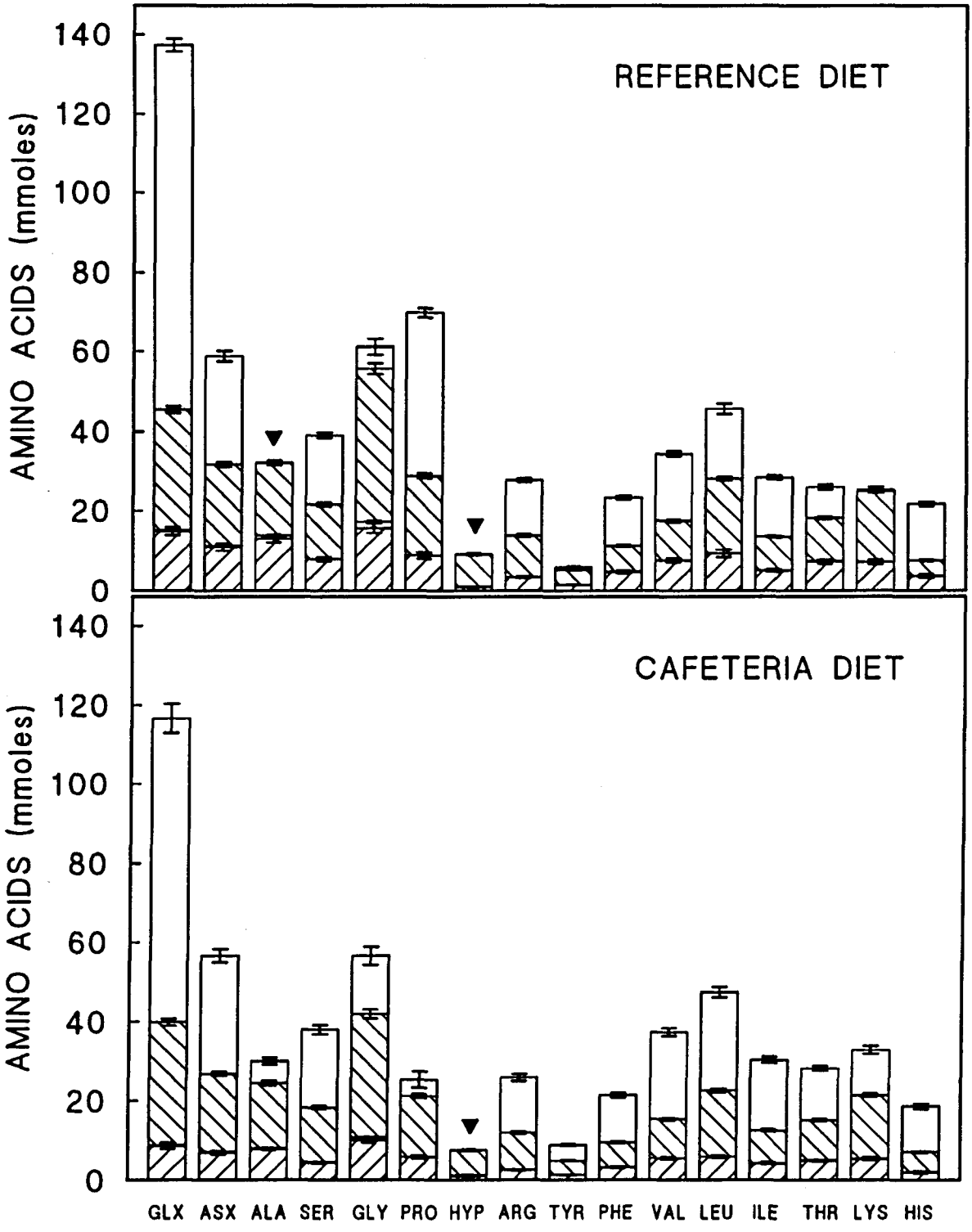
Figure 3 — AMINO ACID CONTENT IN THE BODY OF 30, 45 AND 60 DAY RATS FED EITHER A REFERENCE OR A CAFETERIA DIET.
Upper panel: reference diet, lower panel: cafeteria diet. The highest (white) bars correspond to 60-day rats, the dashed bars to 45-day rats and the crossed (lower) bars to the 30-day rats. The data presented are the mean ± SEM of six different animals per group
*Statistical comparison of the data (two-way ANOVA): The effect of **time** is significant (P<0.05) for all amino acids. The effect of diet is significant [P values between brackets] only for Glx [0.0163], Ala [0.0046], Ser [0.0000], Tyr [0.0000], Phe [0.0017], Val [0.0026], Ile [0.0000] and His [0.0000], and non-significant [P>0.05] for all others.*

Figure 4 — FATE OF DIETARY AMINO ACIDS IN RATS FED THE REFERENCE OR CAFETERIA DIETS.
Upper panel: reference diet, lower panel: cafeteria diet. Each value is the mean ± SEM of six different animals per group. The data presented refer to the whole 30-60 day period. The columns are stacked. The total height of columns represents the amount of each amino acid present in the diet ingested. The bottom —dashed [///]— part of the columns corresponds to the amino acid content of the droppings (i.e. not absorbed); the next very small portion, limited to a thin line between both crossed sections —at the scale of the figure— corresponds to the amino acids lost in the urine; the next —dashed [\\\]— part is the amount of each amino acid accrued in the body of the rat; the upper —white— segment of the column is the amount of amino acid purportedly oxidized or transformed. An inverted triangle [▾] indicates the amino acids for which the sum of non-absorbed, excreted and accrued amino acid was higher than the intake.
*Statistical comparison between of the data (two-way ANOVAs) [the differences for non absorbed and urine excreted amino acids are presented in Figure 3 and Table 3]. The overall effect of **diet** on **ingested** [P=0.0000], **metabolized** [P=0.0000], **accrued** [P=0.0000], lost in **urine** [P=0.0001] or **not absorbed** [P=0.0000] were significant. As for individual amino acids, the effects of diet gave significant differences (P<0.05, Tuckey test) for ingested Glx, Asx, Ala, Ser, Gly and Pro; metabolized Glx, Ala, Gly, Leu and Lys; accrued Ala, Gly and Pro; urinary Ala and Gly; and not-absorbed Glx, Asx, Ala, Ser, Gly, Pro and Leu.*


PERCENT AMINO ACIDS
20
15
10
5
0
GLX
ASX
ALA
SER
GLY
PRO
HYP
ARG
TYR
PHE
VAL
LEU
ILE
THR
LYS
HIS


AMINO ACID INGESTED
NOT ABSORBED (%)
45
40
35
30
25
20
15
10
5
0
GLX
ASX
ALA
SER
GLY
PRO
HYP
ARG
TYR
PHE
VAL
LEU
ILE
THR
LYS
HIS


REFERENCE DIET
CAFETERIA DIET
AMINO ACID CONTENT (mmoles)
AMINO ACID CONTENT (mmoles)
0
10
20
30
40
50
60
GLX
ASX
ALA
SER
GLY
PRO
HYP
ARG
TYR
PHE
VAL
LEU
ILE
THR
LYS
HIS


REFERENCE DIET
CAFETERIA DIET
AMINO ACIDS (mmoles)
AMINO ACIDS (mmoles)
0
20
40
60
80
100
120
140
GLX
ASX
ALA
SER
GLY
PRO
HYP
ARG
TYR
PHE
VAL
LEU
ILE
THR
LYS
HIS

treball enviat per publicar a INTERNATIONAL JOURNAL OF OBESITY

Individual amino acid balances in young lean and obese Zucker rats fed a cafeteria diet.

I. Rafecas, M. Esteve, J.A. Fernández-López, X. Remesar and M. Alemany

Departament de Bioquímica i Fisiologia, Facultat de Biologia, Universitat de Barcelona, Barcelona Spain.

SUMMARY

The amino acid composition of the diet ingested by reference and cafeteria diet–fed lean and obese Zucker rats has been analyzed from day 30 to 60 after birth. Their body protein amino acid composition was measured as well as the urinary and faecal losses incurred during the period studied. The protein actually selected by the rats fed the cafeteria diet had essentially the same amino acid composition as the reference diet. The mean protein amino acid composition of the rat showed only small changes with breed, age or diet.

Cafeteria-fed rats had a higher dietary protein digestion/absorption efficiency than reference diet-fed rats. Obese rats wasted a high proportion of dietary amino acids when given the reference diet, but not on the cafeteria diet. In all cases, the amino acids lost as such in the urine were a minimal portion of available amino acids.

In addition to breed, the rates of protein accretion are deeply influenced by diet, but even more by the age —or size— of the animals: cafeteria-fed rats grew faster, to higher body protein settings, but later protein accrual decreased considerably; this is probably due to a limitation in the "blueprint for growth" which restricts net protein deposition when a certain body size is attained. Obese rats, however, kept accruing protein with high rates throughout.

Diet composition —and not protein availability or quality— induced deep changes in amino acid metabolism. Since the differences in the absolute levels of dietary protein or carbohydrate energy ingested by rats fed the reference or cafeteria diets were small, it can be assumed that high (lipid) energy elicits the changes observed in amino acid metabolism by the cafeteria diet. The effects induced in the fate of the nitrogen ingested were more related to the fractional protein energy proportion than to its absolute values. Cafeteria-fed rats tended to absorb more amino acids and preserve them more efficiently; these effects were shown even under conditions of genetic obesity.

There were deep differences in handling of dietary amino acids by dietary or genetically obese rats. The former manage to extract and accrue larger proportions of their dietary amino acids than the latter. The effects of both "models" of amino acid management were largely additive, suggesting that the mechanisms underlying the development of obesity did not run in parallel to those affecting the control of amino acid utilization. Obesity may be developed in both cases despite a completely different strategy of amino acid assimilation, accrual and utilization.

INTRODUCTION

Obesity affects the management of dietary

nitrogen in different ways depending on the origin of the obesity. Hyperphagia-induced models, such as the cafeteria diet administration, often result in the development of amino nitrogen sparing mechanisms, such as lower urea cycle enzyme activities (1), that result in higher carcass retention of N (2) and increased amino acid availability (3,4). On the other hand, the genetically obese Zucker fa/fa rats do not show altered urea production / nitrogen excretion (5), a situation coexisting with an increased protein accumulation related to extreme fat storage (6). The effects of cafeteria diet–induced hyperphagia on genetically obese rats result in additive effects with respect to N management, since the amino acid sparing effect of the excess energy available in some way counteracts the nitrogen-wasting effect of genetic obesity (5).

Most studies on nitrogen retention and efficiency of deposition have centred on the effects of diet protein on growth, balances or other health topics (7). The relative scarcity of certain individual amino acids in the diet is partly counteracted by an increase in their intestinal absorption (8) and tissue reutilization, with lower oxidation and urea excretion (9,10).

When protein constitutes a large proportion of the diet energy, especially under conditions of low energy availability, like starvation or dieting (11), much of this dietary protein is used for energy, which results in negative N balance (12), lowered protein synthesis and —very often— net loss of body protein (13). The dietary excess of amino nitrogen when there energy substrate availability is limited, necessarily involves the oxidation of part of these amino acids for energy.

This study has been developed as a strict balance analysis in order to determine the differences —if any— in net amino acid availability and protein synthesis induced by the combined effect of an energy–rich cafeteria diet on genetically obese animals, as well as the eventual influence of diet amino acid composition on the N management.

MATERIALS AND METHODS

Animals and diets

The animals studied were obese (fa/fa) or lean (Fa/?) female Zucker rats, bred at the University of Barcelona Animal Service from Charles River France heterozygous parents; they were used 30 days after birth (weaned on day 22). The rats were housed individually, either in polypropylene-bottomed cages with wood shavings as absorbent material, or in polycarbonate metabolic cages (Tecniplast Gazzada, Italy) which allowed the daily estimation of the consumption of individual food items. The cages were maintained in a light (on from 08.00 to 20.00), humidity (70-80 % relative humidity) and temperature (21-22 °C) controlled environment.

series of both lean and obese rats were fed a commercial reference pellet diet (type A04 from Panlab, Barcelona, containing 170 g/kg protein, 587 g/kg digestible carbohydrate, and 30 g/kg lipids) and tap water *ad libitum*. All studies involving the measurement of food intake were carried out with the rats kept individually in metabolic cages. The animals receiving the cafeteria diet were presented daily with a fresh offering of biscuits spread with liver pâté, bacon, banana, chow pellets (as indicated above), tap water and whole milk complemented with 333 g/l sucrose plus 10 g/l of a mineral and vitamin supplement (Gevral, Cyanamid Ibérica) (14). All the materials were previously weighed and presented in excess (c. 20-30 % higher than the expected consumption). Twenty-four hours later, the remaining debris was isolated, identified and weighed. The drying of food leftovers was corrected for by determining the amount of water lost in one day from known weight food samples left in a cage with no rats. This diet is a simplified version of an earlier cafeteria diet developed and studied

by us (14), scaled down by selecting only the items actually consumed in significant portions. The animals were weighed every day at the same hour (11.00 to 12.00).

Experimental setup

For each breed (lean or obese) and dietary group (reference– or cafeteria–fed) three sets (5-7 each) of animals were studied, being killed at 30 (0 days of dietary treatment), 45 (15 days of treatment) or 60 (30 days of treatment) days after birth. The 60–day group was used for daily individual diet intake analysis (metabolic cages) for the whole 30 days of the study. The other rats (45–day, and 30–day old) were kept individually in ordinary cages, where they were fed the same diets; their intake was not measured.

On days 30, 45 or 60, the allotted groups of rats were weighed and immediately killed by decapitation. Their corpses were again weighed (the difference being the net loss of blood and fluids) and then dissected. The contents of their intestine were carefully removed and weighed. The weights recorded and used for calculations were the empty body weights. The remaining carcass was then minced and ground with a blender, sampled and stored at –20 °C until processing.

Analytical procedures

The ground carcasses were sampled following a previously tested sampling protocol (5). The samples were repeatedly minced to a smooth paste with a blender; this was used for further homogenization and analysis. The constituents of the diets given to the animals were also ground and homogenized, and then subjected to the same analytical procedures. Blood samples were also analyzed and used to correct the data obtained for the different groups accounting for the blood lost during the killing and dissecting process.

The rat tissue samples, droppings and food items were further homogenized with a Politron homogenizer, then their proteins —as well as those in the urine samples— were hydrolysed with 6 N HCl in sealed glass tubes under nitrogen at 105 °C for 48 hours (15). The hydrolysates were cleaned by ultrafiltration, neutralized, and their phenyl-hydantoines were prepared (16). All series of determinations included standards of known amino acid mixtures. Prior to derivatization, an internal standard of norleucine was also added to each sample. The amino acid analyses were carried out in an LKB 2150 high performance liquid chromatograph using Spherisorb ODS-2 (5 μm) columns (150x4 mm) and a gradient of ammonium acetate/acetonitrile/methanol as mobile phase at 60 °C (16).

The losses due to the formation of Maillard adducts (17), acid decomposition, and the efficiency of the derivatization process were corrected for by means of the internal standards, series of standards run with each batch of samples and the final adjustment of the sample composition to the content of nitrogen in the sample, measured with a Carlo Erba NA-1500 elemental analyzer. Since the oxidation of –SH groups was higher than expected, and the recoveries of cysteine and methionine poor, these data, together with those of tryptophan —destroyed in the acid hydrolysis procedure— were not included in the tabulated results. All references to "total" amino acids exclude these non–measured amino acids. Each batch of each food item used in the experiments was analyzed independently. The data given in Table 1 are mean food composition values, but in all cases the composition data of the actual food batches were used for calculations. No statistically significant deviations of composition in any of the food items were found.

Calculations and statistics

The analyses of body composition of the series of rats killed on days 30 and 45 were used for the calculation of the composition of the rats kept in metabolic cages (killed at day 60, after one month of daily measurement of intake and body weight) on days 30 and 45.

Since their weights were very close ($P > 0.95$, Student's t test) for all pairs tested, and the feeding scheme was the same, it was assumed that the percentage composition of the series of rats compared was identical for matching age and diet.

The amino acid composition of the samples, and the mass of the rat paste (and that of the blood shed) allowed the estimation of the amount of each amino acid present in each of the two age-groups studied, and allowed the quantitative estimation of the differences between the amino acids stored, always related to the actual empty body weight of the animal referred to the age of comparison.

The known amount of each food item consumed daily by each rat (measured in the rats kept for 30 days in metabolic cages) and its amino acid composition (Table 1) were used to establish the amount of each amino acid ingested by each rat for each day. The tabulated data for each diet component were combined in order to determine the gross intake of each amino acid during both 15-day periods studied. Statistical comparisons between the groups were made with a standard two– or three–way ANOVA program, as well as with the Student's t and the χ^2 test.

RESULTS

Table 2 shows the energy components of the diets actually eaten in 30 days by lean and obese rats. Obese rats ingested more energy, protein, lipid and carbohydrate than their lean counterparts. Cafeteria-fed rats ingested more energy, protein and lipid than the rats fed the reference diet. The energy derived from protein and carbohydrate was lower, and that from lipid higher, in cafeteria diet-fed rats than in the animals fed the reference diet. The diets actually selected by obese rats receiving the cafeteria diet were not different from those chosen by lean rats as to amino acid composition. This composition was —again— not statistically different from that of the reference diet protein as shown in Figure 1.

Figure 2 presents the proportion of dietary amino acids ingested but not absorbed (and thus excreted in the droppings). Lean rats fed the reference diet absorbed a mean 82 % of the diet amino acids, whilst obese rats absorbed somewhat less: a mean 77 %. The figures were higher in both cases when referred to rats fed the cafeteria diet: 89 % and 88 % as mean values for lean and obese rats. The effects of breed were not statistically significant for most amino acids, but the effects of diet were more marked and affected practically all amino acids. The amino acids absorbed with the highest efficiency were Glu + Gln (Glx), Pro, Arg and His, and those present in the highest proportion in the droppings were Ala, Hyp, Thr and Lys.

The amino acid composition of lean and obese rats on days 30, 45 and 60 are shown in Figure 3. Obesity resulted in a higher deposition of most amino acids, in rats receiving the reference or the cafeteria diets. Cafeteria feeding also induced a higher deposition of amino acids in both breeds of rats. The amount of each amino acid present in the body of the 30–day rats was similar for lean and obese rats. On day 45, the differences between groups were more marked, being maximal on day 60. The most abundant amino acid was Gly, followed by other non-essential amino acids (Glu + Gln, Ala, Pro). The pattern of amino acid composition changed little with either age, diet or breed; the most marked deviation from this pattern was found —for 45-60 day rats— in the relatively higher differential deposition of essential amino acids in lean rats fed the cafeteria diet as compared with non-essential amino acids (namely Phe, Val, Leu, Ile, Thr and Lys compared with Glu + Gln, Asp, Ser, Gly and Pro). This same trend is observed —albeit less marked— in the other three groups.

Table 3 presents the mean daily rates of amino acid incorporation into body protein

calculated from the data shown in Figure 3. The rates of net protein synthesis were coincident, in the 30-45 days period, for reference diet–fed lean and obese rats, and higher —but also similar for both breeds— for cafeteria–fed rats. In the second 15-day period, however, there was a marked decrease in the net protein deposition rates for lean rats, steeper for cafeteria–fed animals than for reference-fed controls. Obese rats, however, maintained (reference diet) or partially slowed (cafeteria diet) their net protein synthesis rates, in a very dissimilar pattern from that of lean rats. As a rule, the cafeteria diet resulted in a heavy accrual of amino acids in the first 15 days of the experiment, which resulted in larger 45-day amino acid pools in cafeteria–fed rats than in controls under the reference diet. This effect tapered off in the second 15-day stretch, when the rats fed the reference diet diminished their pace of protein deposition but not so heavily. Obese rats, on the other hand, kept stocking their protein pools beyond this 45-day mark, at a high rate, relatively unaffected by diet

The fate of the amino acids ingested by lean and obese rats is shown —in a stacked column presentation— in Figure 4. The rats fed a reference diet ingested a similar (lean) or much higher (obese) amount of amino acids than the rats fed the cafeteria diet. Obese rats ate more amino acids than their lean counterparts, the differences being significant for all amino acids regardless of the diet ingested. Lean rats, however did not show significant effects of diet on individual amino acid intake except for certain amino acids, whereas obese rats showed significantly higher intakes for all amino acids except Lys when fed the reference diet.

Urinary losses of amino acids were very small as compared with the intake, being practically zero in all groups for many amino acids, especially Tyr and Ile, and showing only small —albeit measurable— amounts for Ala and Hyp. In no case was amino acid excreted in amounts higher than 1.5 mmoles (Ala) in 30 days.

The proportion of each amino acid ingested and not accrued nor lost (faeces, urine) was widely variable, being more dependent on the specific amino acid than on diet or breed; the lowest values were found for Hyp, which was lost or accrued in higher proportion than that ingested. The portion of amino acid not accrued and available for transformation or metabolic disposal was maximal for Glu + Gln and minimal for Ala, Gly and Tyr in addition to Hyp.

The minimum metabolized amino acids in the two 15-day periods studied are shown in Table 4. The effects of time were very marked, the metabolized amino acid share increasing in the second 15-day stretch as compared with the first in lean rats. The extent of change was wider for cafeteria rats than for reference-fed: from lower 30-45 day values to a similar proportion of ingested amino acids in the 45-60 days period. Obese rats behaved differently, since those fed the reference diet maintained —in general terms— the same proportion of amino acids metabolized and not accrued for both periods. However, the fa/fa rats fed the cafeteria diet showed higher rates of amino acid utilization for most amino acids during the second 15-day period..

DISCUSSION

It has been often claimed that the self-selected diets are not uniform in terms of energy, amino acid and micronutrient content (18). However, the amino acid percentage composition of the diet selected by cafeteria rats was practically identical to that of the reference diet, the composition of which is a standard formulated long ago by laboratory rat breeders as the most suitable for growth and maintenance of the rat (19).

The rat can control its intake very well

(20), so it eats varying amounts of food with different energy densities in order to obtain the energy and amino acids (21) it needs for growth and maintenance. Although some of its more usual components contain protein of high biological quality, it has been suggested that the cafeteria diets can provide an insufficient amount of protein (22). The rats receiving a cafeteria diet select a mean diet consisting of similar amounts of protein and carbohydrate as ingested with standard diets, but they eat a much greater proportion of fats (14,23).

The present study was formulated as a comparison of the effects of a purportedly nutrient-rich cafeteria diet (22,23) against a standard diet on genetically obese versus lean rats. However, the close similitude between amino acid composition of the diets and the relative similar amount of protein ingested has allowed us —in addition— to compare the effects of diet energy level alone on nitrogen handling, since both diets are comparable in terms of protein amount —but not proportion— and quality. In this way, we can compare the fate of each individual amino acid ingested in a similar proportion from two diets, with different levels of energy but with a uniform protein fraction. This setting has the additional advantage that in either case the rats fed themselves *ad libitum*, without other constrictions.

Cafeteria-fed rats had a higher dietary protein digestion/absorption efficiency than reference diet-fed animals. This resulted in increased availability of dietary amino acids, and can help to explain their high circulating levels (4), as well as the sustained rates of N retention (1) induced by the cafeteria diets. Obese rats wasted even higher proportions of dietary amino acids when given the reference diet. However, their ability to extract amino acids from the cafeteria diet was similar to that of their lean counterparts, despite their somewhat higher share of many essential amino acids. The rates of absorption for the different amino acids were not uniform, probably as a consequence of diet-induced changes in the intestinal amino acid transport systems. This is in agreement with the modulation of intestinal amino acid transport by energy availability (24) and physiological condition (25,26).

In the rat, both genetic obesity and cafeteria feeding result in higher body weight and increased fat deposition (27). However, rats with genetic obesity usually attain much higher fat stores since they have a defective thermogenic system (28), exactly the opposite of cafeteria rats, which show increased heat production rates (29). Zucker fa/fa rats are remarkably efficient in their utilization of dietary energy (30) in contrast with cafeteria rats, which waste most of the energy ingested, and store only a fraction of it (27). This scheme contrasts with their handling of dietary nitrogen: cafeteria rats limit urea production (1), absorb a larger share of diet amino acids and show high circulating levels of amino acids (3,4), whereas, obese rats excrete important amounts of nitrogen, mainly in the form of urea (5), and extract a lower proportion of diet amino acids than lean rats when fed the reference diet. Since obese rats eat large amounts of food, there remains enough amino acid nitrogen for protein deposition and lean body mass increases, paralleling —although with lower deposition rates— fat deposition (31,32).

In addition to breed, the rates of protein accretion are deeply influenced by diet, but even more by the age —or perhaps size— of the animals: cafeteria-fed rats grew faster, to higher body protein settings. Later on, net protein deposition rates in lean rats slowed more steeply than in rats fed the reference diet. Obese rats fed the reference diet kept accruing protein with practically unaltered rates. Those receiving the cafeteria diet initially deposited more protein, but later on lowered their rate to the standard pace of rats fed the reference diet. This is in agreement with the general tendency to limit net protein accretion with increasing age (33).

The protein accretion in cafeteria rats, on the other hand is higher, in part because of high energy and amino acid availability, a consequence of the amino acid preservation schemes outlined above; but this fast growth rapidly tapers off. This is probably due to a limitation in the "blueprint for growth", which restricts net protein deposition when a certain body size is attained. Curiously, this limitation is not observed —at least for the period studied— in fa/fa rats, which continue accruing protein at practically unchanged rates. These results are consistent with the existence of different ceilings for fat and protein content of lean and obese Zucker rats (33). The slowing pace of protein deposition rates with age —and size— (31,32) is evident even when the rates are expressed as a percentage of the total protein mass of the rat. It cannot be attributed to lowered circulating amino acid levels (34), nor to lower amino acid availability, since the proportion of all amino acids absorbed —and thus available for protein deposition or other metabolic uses— remained high in all cases.

The very small proportion of amino acids lost as such in the urine could not significantly influence the amino acid balance. Rats fed a cafeteria diet excrete less (35) nitrogen than the animals fed standard diets; however, their nitrogen balances present much wider unaccounted for nitrogen gaps (5). The data observed here for both obese and lean rats may give support to the existence of such a gap when the slowing of accretion rates for the 45-60 day period is combined with the high net dietary amino acid availability. The intestinal absorption was higher than for the reference diet and this nitrogen was not excreted through the urine, which is fully in agreement with earlier data in which only total nitrogen balance was measured (1).

The mean protein amino acid composition of the rat showed only small changes with breed, age or diet, but some amino acids were incorporated in a somewhat larger proportion into the protein of elder rats; in lean and obese rats fed the cafeteria diet most of the essential amino acids showed a larger change in content from 45 to 60 days than non-essential amino acids, suggesting a shift in the overall composition of the rat protein. This may be a consequence of the synthesis of specific proteins, not of generalized growth. However, the changes in mean protein amino acids were small, maintaining a broadly uniform rat protein composition in spite of breed, diet and age differences.

The presence of fibre in the reference diet (4.3 % of dry weight) is not too far away from the calculated fibre content in the mean cafeteria diet ingested by the rats (about 3 % of dry weight), but it can affect —at least— the digestion and absorption interval (36).

Obese rats ate more protein and carbohydrate when fed the standard reference diet than when offered the cafeteria diet, but the lipid content was much smaller in the former than in the latter, so that obese rats fed the cafeteria diet in fact ingested more energy than those receiving the reference diet. In all cases (cafeteria diet, genetic obesity) the excess energy intake did not actually affect the appetite for protein nor its ingestion, which has been postulated to be set by an independent mechanism other than energy intake (37). Then, the lean rats fed cafeteria diet ate essentially the same protein as those fed on the reference diet (14), but they absorbed it better, accrued more and had more amino acids available for other metabolic uses than the rats fed the reference diet, despite the latter being richer in protein. Obese rats, on the other hand, showed a comparable situation, but a higher intake (energy, protein) setting than lean rats. Obese rats fed the cafeteria diet, however, ingested an even lower proportion of protein than lean rats, and thus the differences versus reference diet-fed rats were higher.

The most striking difference between the two diets as to amino acid management is found in the deep changes induced in amino

acid metabolism by diet composition: not by protein availability or quality —which was essentially identical for both diets tested—, but by the other components of the diet or the ratio of protein content versus other energy substrates. The diets actually taken up by the four groups of rats studied had a different proportion of protein–derivable energy (21.7 % for reference diet and 14.9 % / 13.5 % for lean / obese cafeteria diet–fed rats). Since the difference in the levels of energy derivable from protein in the diet ingested was small for lean rats fed the reference or cafeteria diets —as it was for carbohydrate, but not for the lipid fraction—, it can be assumed that it is the excess (lipid) energy that elicits the changes observed in amino acid metabolism by the cafeteria diet in lean rats.

The levels of protein available in either case were higher than the limiting values found for protein-caloric malnutrition (38) and other protein-deficient situations. In addition, the level of protein intake was enough in any case for sustained growth and maintenance —as demonstrated by the increases observed in weight and protein content. The effects observed in the fate of the nitrogen ingested were more related to the fractional *protein energy* proportion than to its absolute values. Cafeteria-fed rats tended to absorb more and preserve better their amino nitrogen, as observed elsewhere (1), and these effects were shown even under conditions of genetic obesity. In either case, the saving of amino acids was parallel to that found in other situations where there was a real —not relative, as in the case discussed— scarcity of amino acids, with a similar onset of protein-sparing mechanisms: starvation (39) or diluted diets (40). In all these cases, amino acid oxidation is low, with decreased urea production (10); but circulating amino acids are maintained within acceptable limits through limited proteolysis (41). Since there is a conflict between the setting of amino acid sparing mechanisms and their increased availability derived form the ingestion of comparable amounts of amino acids and the higher effectiveness of the saving mechanisms, the only outlets available for these amino acids are a higher accrual and the essentially unknown mechanisms defined as nitrogen gap (5).

Another conclusion derived from this study is the deep difference in handling of dietary amino acids shown by dietary or genetically obese rats. The former manage to extract and accrue larger proportions of their dietary amino acids than the latter. The effects of both "models" of amino acid management were largely additive, suggesting that the mechanisms underlying the development of obesity did not run in parallel to those affecting the control of amino acid utilization. Obesity may be developed in both cases despite a completely different strategy of amino acid extraction from the diet, accrual and utilization from a diet of essentially the same composition but with a different proportion of protein.

ACKNOWLEDGEMENTS

Work supported by grant PB88-0208 from the 'Dirección General de Investigación Científica y Técnica' from the Government of Spain. The authors acknowledge the technical assistance of Maria Reixach, Dr. Isidre Canals and Pilar Fernández from the Scientific and Technical Services of the University of Barcelona and their help with the aminoacid analyses. Thanks are given to Robin Rycroft for his assistance in the edition of the manuscript.

REFERENCES

1 Barber T, Viña JR, Viña J, Cabo J. Decreased urea synthesis in cafeteria–diet induced obesity in rat. Biochem J 1985; **230:** 675-681.

2 Barr HG, McCracken KJ. High efficiency of energy utilization in "cafeteria" and force fed–rats left at 29 °C.Br J Nutr 1984; **51:** 379-387.

3 Calles-Escandon J, Cunningham J, Felig P. The plasma amino acid response to cafeteria feeding i the rat: influence of hyperphagia sucrose intake and

exercise. Metabolism 1984; **33:** 364-368.
4 Rafecas I, Esteve M, Remesar X, Alemany M. Plasma amino acids of lean and obese Zucker rats subjected to a cafeteria diet. Biochem Internat 1991; **25:** 797-806.
5 Esteve M, Rafecas I, Remesar X, Alemany M. Nitrogen balances of lean and obese Zucker rats subjected to a cafeteria diet. Int J Obesity 1992; **16:** in the press.
6 Bray GA, York DA. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. Physiol Rev 1979; **59;** 719-809.
7 Kielanowski J. Estimates of the energy cost of protein deposition in growing animals In: Blaxter KL ed. Energy metabolism. London: Academic Press. 1965; 13-19.
8 Wapnir RA, Lifshitz F. Absorption of amino acids in malnourished rats. J Nutr 1974; **104:** 843-849.
9 Brookes IM, Owens FN, Garrigus US. Influence of amino acid level in the diet upon amino acid oxidation by the rat. J Nutr 1972; **102:** 27-36.
10 Sapir DG, Walser M. Nitrogen sparing induced early in starvation by infusion of branched-chain ketoacids. Metabolism 1977; **26:** 301-308.
11 Scalfi L, Contaldo F, Borrelli R, De Caterina M, Spagnuolo G, Alfieri R, Mancini M. Protein balance during very-low-calorie diets for the treatment of severe obesity. Ann Nutr Metab 1987; **31:** 154-159.
12 Fisler JS, Drenick EJ, Blumfield DE, Swendseid ME. Nitrogen economy during very low calorie reducing diets: quality and quantity of dietary restriction. Am J Clin Nutr 1982; **35:** 471-486.
13 Tsukahara S, Ohno M, Ikeda Y. Dieting using a very low calorie diet. In: Bray GA, LeBlanc J, Inoue S, Suzuki M, eds. Diet and Obesity. Tokyo/ Basel: Japan Scientific Societies Press/ Karger. 1988; 205-217.
14 Prats E, Monfar M, Castellà J, Iglesias R, Alemany M. Energy intake of rats fed a cafeteria diet. Physiol Behav 1989; **45:** 263-272.
15 Allen G. Sequencing of protein and peptides. In: Burdon RH, van Knippenberg PM, eds. Laboratory Techniques in Biochemistry and Molecular Biology. Amsterdam: Elsevier, 1989; **9:** 40-42.
16 Heinrikson RL, Meredith SC. Amino acid analysis by reverse–phase high–performance liquid chromatography: Precolumn derivatization with phenylisothiocyanate. Anal Biochem 1984; **136:** 65-74.
17 Whistler RL, Daniel JR. Carbohydrates. In: Fennema OR ed. Food Chemistry. New York: Marcel Dekker, 1985; 69-137.
18 Moore B. The cafeteria diet —An inappropriate tool for studies of thermogenesis. J Nutr 1987; **117:** 227-231.
19 Rogers AE. Nutrition In: Baker HJ, Lindsey JR, Weisbroth SH eds. The Laboratory Rat. New York: Academic Press, 1979; **1:** 123-152.
20 Oscai LB, McGarr JA. Evidence that the amount of food consumed in early life fixes appetite in the rat. Am J Physiol 1978; **235:** R141-R144.
21 Cohn C, Joseph D. Influence of body weight and body fat on appetite of normal lean and obese rats. Yale J Biol Med 1962; **34:** 598-601.
22 Sclafani AA, Springer D. Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. Physiol Behav 1976; **17:** 461-471.
23 Naim M, Brand J, Kare MR, Carpenter RG. Energy intake weight gain and fat deposition in rats fed flavored nutritionally controlled diets in a multichoice "cafeteria" design. J Nutr 1985; **115:** 1447-1458.
24 Karasov WH, Solberg DH, Diamond JM. Dependence of intestinal amino acid uptake on dietary protein on amino acid levels. Am J Physiol 1987; **252:** G614-G625.
25 Schedl HP. Intestinal adaptation in diabetes: amino acid and carbohydrate absorption. In: Dowling RH, Riecken OR eds. Intestinal Adaptation, Stuttgart: Schattanes Verlag, 1974; 205-216.
26 Israel Y, Salazar I, Rosenmann E. Inhibitory effects of alcohol on intestinal amino acid transport in vivo and in vitro. J Nutr 1968; **96:** 499-504.
27 Rothwell NJ, Stock MJ. A role for brown adipose tissue in diet–induced thermogenesis. Nature 1979; **281:** 31-35.
28 Triandafillou J, Himms-Hagen J. Brown adipose tissue in genetically obese (fa/fa) rats: exposure to cold and diet. Am J Physiol 1983; **224:** E145-E150.
29 Rothwell NJ, Stock MJ. Brown adipose tissue and diet-induced thermogenesis. In: Trayhurn P, Nicholls D eds. Brown Adipose Tissue. London: Edward Arnold, 1982; 269-298.
30 Keesey RE, Corbett SW. Adjustements in daily energy expenditure to caloric restriction and weight loss by adult obese and lean Zucker rats. Int J Obesity 1990; **14:** 1079-1084.
31 Peret J, Bach AC, Delhomme B, Bois-Joyeux B, Chanez M, Schirardin H. Metabolic effects of high-protein diets in Zucker rats. Metabolism 1984; **33:** 483-492.
32 Radcliffe JD, Webster AJF. Sex, body composition and regulation of food intake during growth in the Zucker rat. Br J Nutr 1978; **39:** 483-492.
33 Dunn MA, Hartsook EW. Comparative amino acid and protein metabolism in obese and non-obese Zucker rats. J Nutr 1980; **110:** 1865-1879.
34 Tsuda TT, Ohkubo T, Kamiguchi H, Tsuda M, Katsunuma T, Tamamura M. Influence of fecal anorexigenic substance (Fs-T) on plasma amino acids in Wistar and Zucker (fa/fa) rats. J Nutr 1989; **119:** 1327-1332.
35 Henry CJR, Rivers JPW, Rayne PR. Reduced obligatory nitrogen loss in rats made obese by cafeteria feeding. Nutr Res 1987; **7:** 1243-1252.
36 Farrell DJ, Girle L, Arthur J. Effects of dietary fibre on the apparent digestibility of major food components and on blood lipids in men Aust J Exp Biol Med Sci 1978; **56:** 469-479.
37 Anderson GH. Control of protein and energy intake: role of plasma amino acids and brain neurotransmitters. Can J Physiol Pharmacol 1979; **57:** 1043-1057.
38 Garlick PJ, Millward DJ, James WPT, Waterlow JC. The effect of protein deposition and starvation on the rate of protein synthesis in tissues of the rat. Biochim Biophys Acta 1975; **414:** 71-84.
39 Cahill GF. Starvation in man. New Engl J Med 1970; **282:** 668-675.
40 Booth DA. Food intake composition for increase or decrease in the protein content of the diet. Behav Biol 1974; **12:** 31-40.
41 Hoffer LJ, Forse RA. Protein metabolic effects of a prolonged fast and hypocaloric refeeding. Am J Physiol 1990; **258:** E832-E840.

Table 1

Amino acid and energy content of the foods offered to cafeteria-fed rats

amino acid	chow pellet	liver pâté	bacon	banana	biscuits	milk
Glx (mmol/kg)	272 ± 20	136 ± 9	184 ± 7	9.4 ± 0.7	98 ± 5	43 ± 5
Asx (mmol/kg)	116 ± 9	84 ± 7	130 ± 5	10.9 ± 0.7	12 ± 1	18 ± 2
Ala (mmol/kg)	60 ± 5	46 ± 5	82 ± 3	5.4 ± 0.7	14 ± 1	8 ± 1
Ser (mmol/kg)	77 ± 7	50 ± 4	71 ± 3	6.3 ± 0.4	19 ± 1	15 ± 2
Gly (mmol/kg)	121 ± 9	100 ± 11	184 ± 10	9.6 ± 0.9	25 ± 1	8 ± 1
Pro (mmol/kg)	138 ± 5	67 ± 5	94 ± 4	5.6 ± 0.6	56 ± 4	26 ± 2
Hyp (mmol/kg)	5 ± 1	15 ± 11	43 ± 3	1.0 ± 0.1	0 ± 0	0 ± 0
Arg (mmol/kg)	55 ± 4	40 ± 4	75 ± 3	5.0 ± 0.3	7 ± 1	6 ± 1
Tyr (mmol/kg)	12 ± 1	18 ± 1	24 ± 1	1.6 ± 0.2	2 ± 0	4 ± 1
Phe (mmol/kg)	46 ± 3	32 ± 2	41 ± 1	3.7 ± 0.3	13 ± 1	8 ± 1
Val (mmol/kg)	68 ± 7	58 ± 4	77 ± 3	5.6 ± 0.4	14 ± 1	15 ± 2
Leu (mmol/kg)	90 ± 7	69 ± 4	92 ± 4	7.6 ± 0.4	24 ± 0	20 ± 3
Ile (mmol/kg)	55 ± 4	43 ± 3	68 ± 2	4.7 ± 0.1	13 ± 1	12 ± 2
Thr (mmol/kg)	51 ± 4	42 ± 4	85 ± 2	6.4 ± 0.9	12 ± 1	11 ± 1
Lys (mmol/kg)	49 ± 4	55 ± 5	97 ± 9	4.7 ± 0.4	4 ± 0	12 ± 1
His (mmol/kg)	43 ± 4	15 ± 1	31 ± 2	7.2 ± 0.9	8 ± 1	7 ± 1
EAA/NEAA ratio[1]	0.47	0.56	0.53	0.73	0.38	0.65
protein (g/kg)	170 ± 7.2	123 ± 13	173 ± 5	14 ± 1	63 ± 1	22 ± 2
carbohydrate (g/kg)	587 ± 20	7.0 ± 2.1	0.0 ± 0.0	171 ± 12	655 ± 34	319 ± 14
lipid (g/kg)	30 ± 1	291 ± 22	299 ± 43	1.0 ± 0.1	155 ± 3	19 ± 3
energy (MJ/g)	14.6	13.5	14.6	3.3	18.5	6.4
protein energy (%)	21.7	17.0	22.1	7.9	6.3	6.4

[1] Essential amino acids [Phe + Val + Leu + Ile + Thr + Lys + His] versus non-essential amino acids [Glx + Asx + Ala + Ser + Gly + Pro + Hyp + Arg + Tyr] ratio

Table 2

Energy composition of the reference and cafeteria diets eaten by lean and obese rats from days 30 to 60

energy derived from:	reference lean	reference obese	cafeteria lean	cafeteria obese	statistical comparison breed	diet
whole diet (MJ/30d)	6.53 ± 0.20	10.90 ± 0.24	8.35 ± 0.19	12.87 ± 0.42	B^R, B^C	D^L, D^O
protein (MJ/30d)	1.42 ± 0.04	2.36 ± 0.05	1.24 ± 0.03	1.74 ± 0.07	B^R, B^C	D^L, D^O
protein (%)	*21.7*	*21.7*	*14.9*	*13.5*		
carbohydrate (MJ/30d)	4.60 ± 0.14	7.67 ± 0.17	4.55 ± 0.18	6.81 ± 0.30	B^R, B^C	
carbohydrate (%)	*70.4*	*70.4*	*53.2*	*52.9*		
lipid (MJ/30d)	0.51 ± 0.01	0.85 ± 0.02	2.65 ± 0.08	4.29 ± 0.16	B^R, B^C	D^L, D^O
lipid (%)	*7.8*	*7.8*	*31.7*	*33.3*		

Statistical comparisons between the groups (Student's *t* test): the letters indicate that there is a significant ($P<0.05$) effect of the given parameter: B = breed and D = diet; superindexed letters refer the significance to the indicated groups: ^{R} = reference diet, ^{C} = cafeteria diet; ^{L} = lean rats, ^{O} = obese rats. Only the significant relations are shown.

Table 3

Mean daily rates of amino acid incorporation into body protein

amino acid	lean reference 30-45	lean reference 45-60	lean cafeteria 30-45	lean cafeteria 45-60	obese reference 30-45	obese reference 45-60	obese cafeteria 30-45	obese cafeteria 45-60
Glx	1089	331	1605	84	922	787	1304	841
Asx	635	270	1131	66	529	705	1033	346
Ala	714	209	921	670	551	732	5436	485
Ser	570	92	753	74	456	412	753	333
Gly	1418	374	2741	90	1578	1373	3067	868
Pro	772	−12	1254	−170	774	402	1062	814
Hyp	309	18	667	−128	301	312	581	232
Arg	320	190	570	159	347	396	458	527
Tyr	93	59	169	69	66	180	120	238
Phe	158	200	648	177	73	281	161	354
Val	261	258	387	207	128	548	543	427
Leu	502	324	213	436	567	462	799	560
Ile	246	225	342	188	215	339	406	351
Thr	381	69	406	180	166	346	468	290
Lys	406	293	598	223	494	302	493	675
His	115	97	174	93	184	58	173	176
total [1]	7.99	3.00	12.58	2.19	7.34	7.60	12.81	7.52
total [2]	5.03	1.24	6.31	0.71	4.82	2.87	6.45	2.14

The data are expressed in μmoles of amino acid residues incorporated into the rat protein per day.
[1] expressed in mmoles of amino acid residues incorporated per day
[2] expressed as a percentage of the rat protein pool.

Table 4

Minimum (balance) amino acids metabolized by rats fed a reference or cafeteria diet

amino acid	age group	lean reference	lean cafeteria	obese reference	obese cafeteria	statistical comparisons breed	diet	time
Glx	**30-45**	**66.3 ± 2.0**	**49.4 ± 0.9**	**82.6 ± 1.0**	**69.5 ± 1.5**	B,B^R,B^C	D,D^L,D^O	T,T^L,T^O,T^R,T^C
	45-60	92.1 ± 0.7	98.2 ± 0.5	88.7 ± 0.3	82.3 ± 0.8			
Asx	**30-45**	**48.2 ± 3.6**	**24.4 ± 0.8**	**73.8 ± 0.9**	**45.2 ± 2.6**	B,B^R,B^C	D,D^L,D^O	T,T^L,T^O,T^R,T^C
	45-60	83.2 ± 1.0	96.9 ± 0.7	72.5 ± 1.0	83.7 ± 0.8			
Ala	**30-45**	**−39.5 ± 10.5**	**−25.5 ± 2.9**	**32.6 ± 3.6**	**−52.2 ± 7.6**	B^R,B^C	D,D^O	T,T^L,T^O,T^R,T^C
	45-60	65.6 ± 3.4	68.6 ± 2.0	22.6 ± 6.3	48.3 ± 3.8			
Ser	**30-45**	**32.1 ± 3.7**	**22.2 ± 1.5**	**66.6 ± 1.0**	**40.6 ± 2.9**	B,B^R	D,D^O	T,T^L,T^O,T^R,T^C
	45-60	91.3 ± 1.3	94.4 ± 0.8	76.8 ± 0.9	78.9 ± 1.0			
Gly	**30-45**	**−14.2 ± 6.4**	**−74.0 ± 2.4**	**21.2 ± 2.7**	**−59.8 ± 7.0**	B^C	D,D^L,D^O	T,T^L,T^O,T^R,T^C
	45-60	76.0 ± 2.0	94.9 ± 1.8	47.1 ± 1.7	59.9 ± 2.2			
Pro	**30-45**	**52.6 ± 2.8**	**25.1 ± 1.4**	**70.9 ± 0.8**	**52.6 ± 2.4**	B^C	D,D^L,D^O	T,T^L,T^O,T^R,T^C
	45-60	100.4 ± 1.1	108.8 ± 0.7	88.6 ± 0.5	67.2 ± 1.4			
Hyp	**30-45**	**−495 ± 33**	**−226 ± 15**	**−283 ± 23**	**−152 ± 22**	B	D,D^L,D^O	T,T^L,T^O,T^R,T^C
	45-60	73.4 ± 11.2	154 ± 5	−212 ± 23	16.3 ± 7.1			
Arg	**30-45**	**49.4 ± 3.5**	**23.9 ± 0.4**	**66.8 ± 1.0**	**50.0 ± 2.2**	B^R	D,D^L,D^O	T,T^L,T^R,T^C
	45-60	77.1 ± 1.2	89.9 ± 0.8	71.6 ± 0.7	50.9 ± 1.8			
Tyr	**30-45**	**25.3 ± 5.5**	**30.6 ± 8.9**	**68.1 ± 1.6**	**60.0 ± 2.3**	B^C	D,D^L	T,T^L,T^O,T^C
	45-60	61.3 ± 1.7	78.2 ± 0.7	28.3 ± 3.4	32.9 ± 2.4			
Phe	**30-45**	**67.7 ± 2.8**	**62.3 ± 0.6**	**91.5 ± 0.9**	**77.2 ± 1.4**	B,B^R	D,D^O	T,T^L,T^O,T^R,T^C
	45-60	70.2 ± 0.7	74.9 ± 0.6	73.8 ± 0.9	56.5 ± 1.7			
Val	**30-45**	**62.0 ± 3.6**	**60.9 ± 0.7**	**88.5 ± 0.8**	**53.6 ± 2.2**	B,B^C	D,D^L,D^O	T,T^L,T^O,T^R,T^C
	45-60	72.4 ± 1.1	83.9 ± 0.6	62.3 ± 1.9	69.1 ± 1.1			
Leu	**30-45**	**48.0 ± 3.9**	**47.8 ± 0.9**	**64.6 ± 0.9**	**49.5 ± 2.3**	B,B^R	D,D^O	T,T^L,T^O,T^R,T^C
	45-60	75.6 ± 1.4	72.7 ± 0.7	77.6 ± 0.9	69.3 ± 1.1			
Ile	**30-45**	**58.7 ± 3.5**	**58.6 ± 0.5**	**77.8 ± 0.6**	**58.8 ± 1.9**	B^R,B^C	D,D^L,D^O	T,T^L,T^O,T^R,T^C
	45-60	71.4 ± 1.0	82.4 ± 0.5	72.3 ± 1.2	69.8 ± 1.0			
Thr	**30-45**	**23.6 ± 4.5**	**38.8 ± 1.5**	**79.0 ± 1.0**	**47.0 ± 2.5**	B,B^R	D,D^O	T,T^L,T^O,T^R,T^C
	45-60	88.9 ± 1.6	79.7 ± 1.0	67.4 ± 2.1	71.6 ± 0.9			
Lys	**30-45**	**17.1 ± 6.2**	**31.2 ± 1.2**	**36.0 ± 2.0**	**45.0 ± 3.1**	B^R,B^C	D,D^L,D^O	T,T^L,T^O,T^R,T^C
	45-60	53.5 ± 2.2	80.5 ± 0.8	69.2 ± 1.3	39.0 ± 2.4			
His	**30-45**	**77.2 ± 2.0**	**63.4 ± 1.0**	**77.2 ± 0.9**	**71.1 ± 1.3**	B^R	D,D^L,D^O	T,T^L,T^O,T^R,T^C
	45-60	85.4 ± 0.7	84.7 ± 0.7	94.5 ± 0.4	73.8 ± 0.9			

The data are expressed as percentage of absorbed (ingested minus lost in the faeces) amino acids. Statistical comparisons between the groups (three-way ANOVA): the letters indicate that there is a significant ($P<0.05$) or highly significant —in *italics*— ($P<0.001$) effect of the given parameter: B = breed, D = diet and T = time; superindexed letters limit the significance to the indicated groups: ^{R} = reference diet, ^{C} = cafeteria diet; ^{L} = lean rats, ^{O} = obese rats; non-superindexed letters refer to the global significance of the effects of breed, diet or time. Only the significant relations are shown. The effects of breed or diet upon individual 15-day time stretches are not shown.

Figure captions

Figure 1

AMINO ACID COMPOSITION OF THE DIETS INGESTED BY ZUCKER LEAN AND OBESE RATS FED A REFERENCE OR A CAFETERIA DIET.

White bars correspond to the reference diet; dashed bars correspond to the cafeteria diet (i.e. the mean composition of the food ingested by cafeteria-fed rats) eaten by lean Zucker rats, and crossed bars correspond to the cafeteria diet eaten by the obese Zucker rats.

Statistical comparison between diets (χ^2 test), i.e. probability of the sets of data being equal: $\chi^2=1.175$, $P=1.0000$ (reference and cafeteria taken by lean rats) and $\chi^2=0.928$, $P=1.0000$ (reference and cafeteria taken by obese rats); (df=15).

Figure 2

PERCENT OF AMINO ACIDS INGESTED BUT NOT ABSORBED BY LEAN AND OBESE ZUCKER RATS FED A REFERENCE OR CAFETERIA DIET.

Upper panel: lean Zucker rats, lower panel: obese Zucker rats. White (higher) columns represent the reference diet, and the dashed columns the cafeteria diet. All data refer to the 30-60 days period, and are presented as mean ± SEM of six different animals per group.

Statistical comparison of the data (two-way ANOVAs)

parameter		glx	asx	ala	ser	gly	pro	hyp	arg	tyr	phe	val	leu	ile	thr	lys	his
BREED	reference							†									
	cafeteria											†		†		‡	†
DIET	lean	†	‡	†	†	†	†	‡	‡	‡	†	‡	‡	‡	†	‡	
	obese	‡	‡	‡	‡	‡	‡	‡	‡	‡	†	‡	‡	‡	‡		

The symbol † indicates a statistically significant ($P<0.05$) difference; the symbol ‡ indicates a higher degree of statistical significance ($P<0.001$) for the differences.

Figure 3

AMINO ACID CONTENT IN THE BODY OF 30, 45 AND 60 DAY LEAN AND OBESE ZUCKER RATS FED A REFERENCE OR CAFETERIA DIET.

Upper panels: lean rats; lower panels: obese rats; left panels: reference diet; right panels: cafeteria diet. The highest (white) bars correspond to 60-day rats, the dashed bars to 45-day rats and the crossed (lower) bars to the younger 30-day rats. The data presented are the mean ± SEM of six different animals per group

Statistical comparison of the data (three-way ANOVAs)

parameter		glx	asx	ala	ser	gly	pro	hyp	arg	tyr	phe	val	leu	ile	thr	lys	his
BREED	reference		†			‡	†	‡	‡	†		†	‡	†	‡		†
	cafeteria			‡	‡	‡	‡	†			‡	‡	‡	‡	‡		

The symbol † indicates a statistically significant ($P<0.05$) difference; the symbol ‡ indicates a higher degree of statistical significance ($P<0.001$) for the differences.

There are statistically significant differences ($P<0.001$) for **all** amino acids on the effects of diet upon either lean or obese rats, as well as for the interaction of time with lean and obese as well as with reference-fed and cafeteria-fed groups.

Figure 4

FATE OF DIETARY AMINO ACIDS IN LEAN AND OBESE ZUCKER RATS FED A REFERENCE OR CAFETERIA DIET.

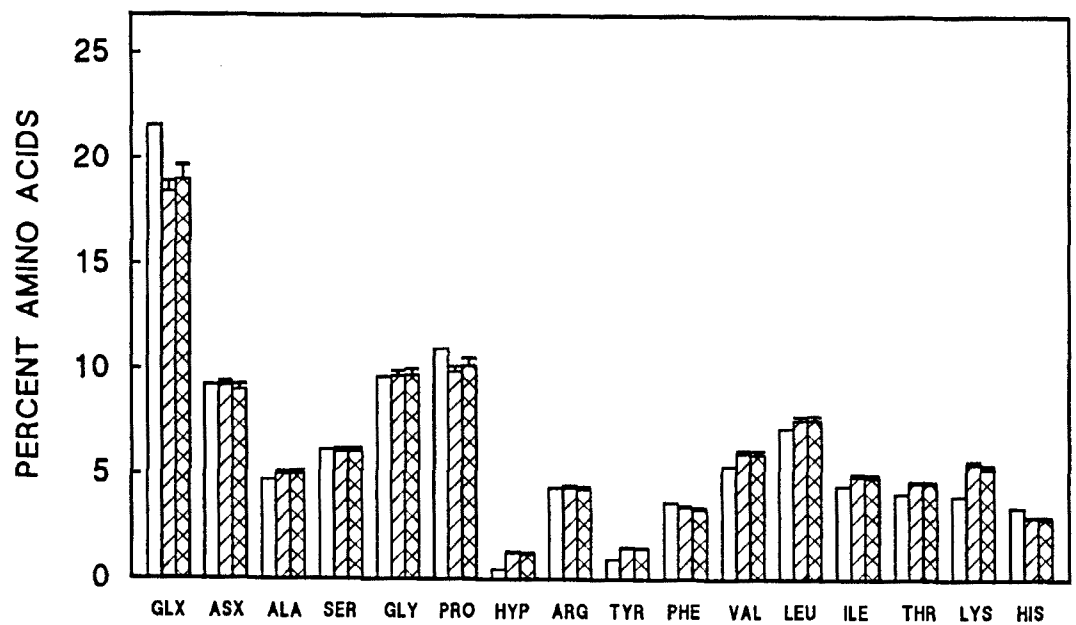
Upper panels: lean rats; lower panels: obese rats; left panels: reference diet; right panels: cafeteria diet. Each value is the mean ± SEM of six different animals per group. The data presented refer to the whole 30-60 day period. The columns are stacked. The total height of columns represents the amount of each amino acid present in the diet ingested. The bottom —dashed [///]— part of the columns corresponds

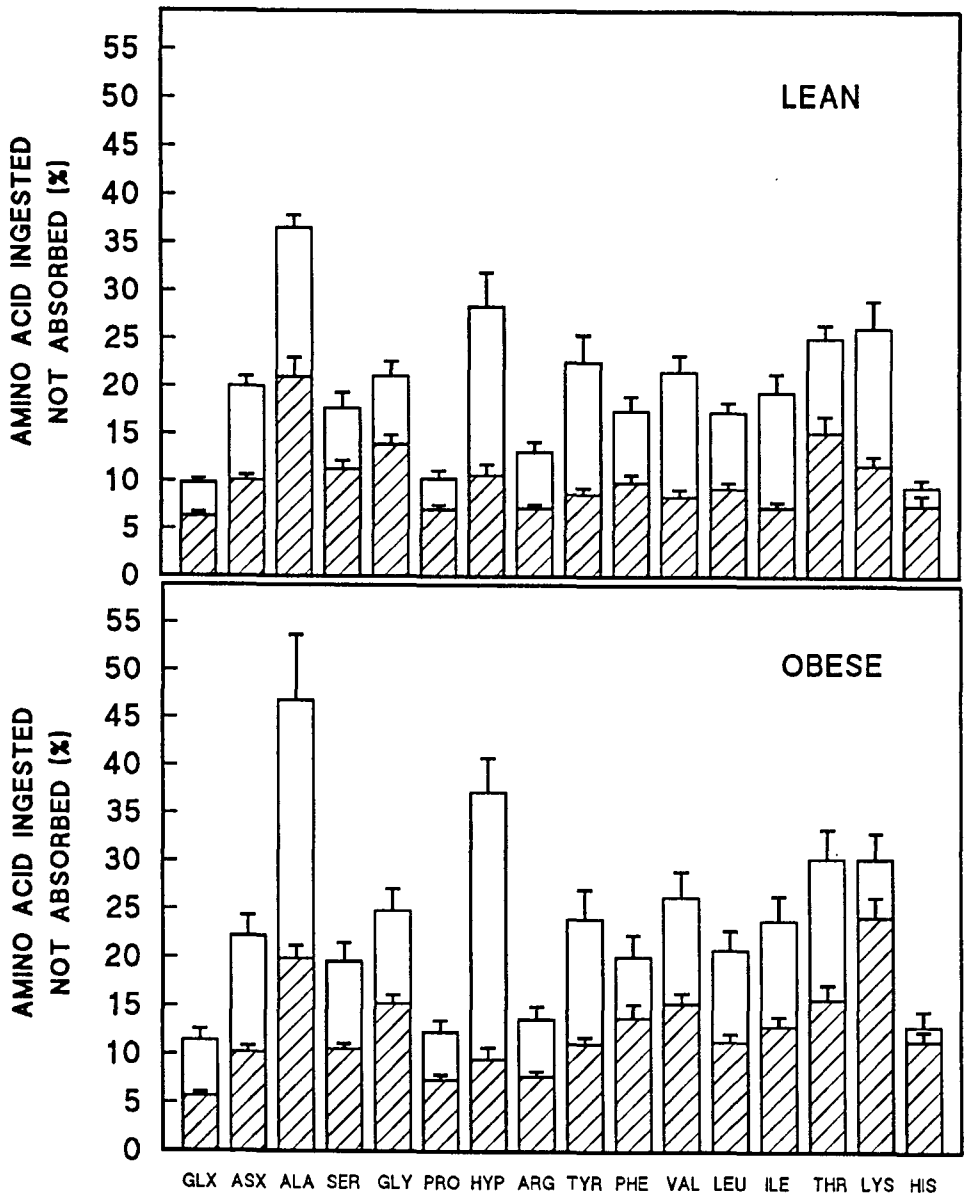
to the amino acid content of the droppings (i.e. not absorbed); the next very small portion —in black— corresponds to the amino acids lost in the urine; the next —dashed [\\\]— part is the amount of each amino acid accrued in the body of the rat; the upper —white— segment of the column is the amount of amino acid purportedly oxidized or transformed. An inverted triangle [▾] indicates the amino acids for which the sum of non-absorbed, excreted and accrued amino acid was higher than the intake.

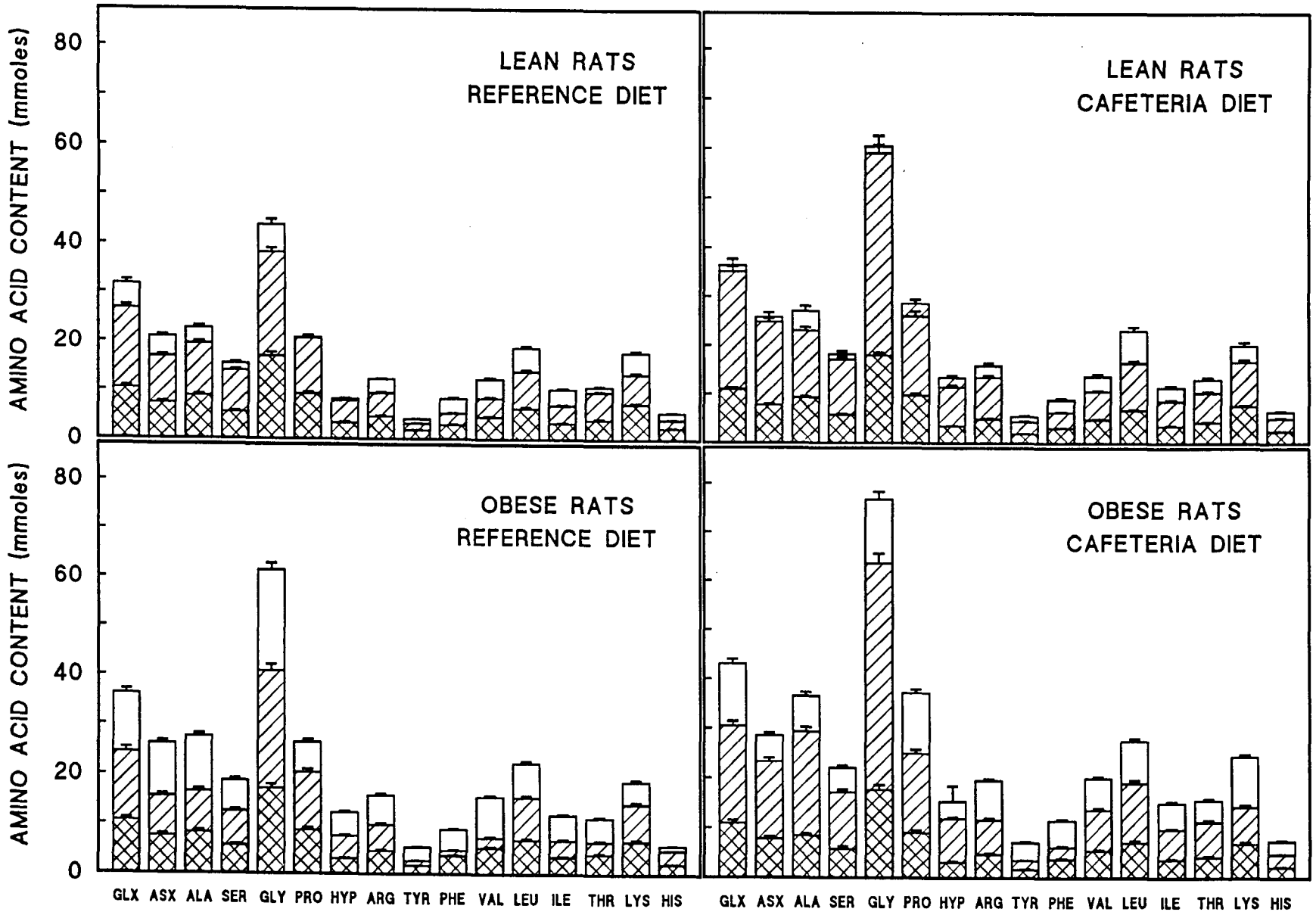
Statistical comparison of the data (three-way ANOVAs)

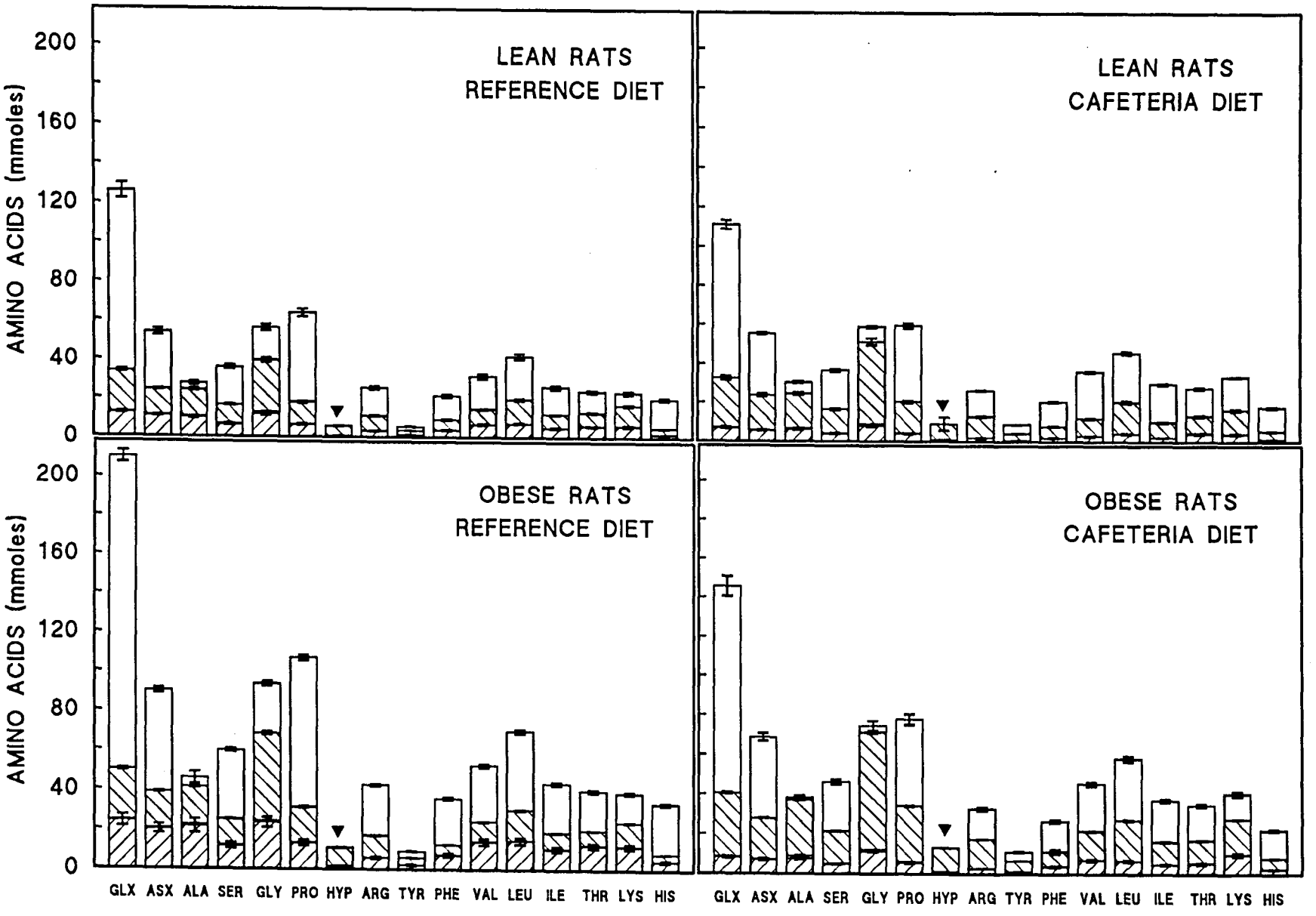
parameter		glx	asx	ala	ser	gly	pro	hyp	arg	tyr	phe	val	leu	ile	thr	lys	his
INGESTED																	
BREED	reference	‡	‡	‡	‡	‡	‡	†	‡	‡	‡	‡	‡	‡	‡	‡	‡
	cafeteria	‡	‡	‡	‡	‡	‡	†	‡	‡	‡	‡	‡	‡	‡	‡	‡
DIET	lean	†						‡		‡		†		†	†	‡	†
	obese	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡		‡
NOT ABSORBED																	
BREED	reference	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡
	cafeteria										†	†		†		‡	†
DIET	lean	†	†		†		†		†		†	†	†	†			
	obese	‡	‡	‡	‡	‡	‡	‡	‡	†	‡	‡	‡	‡	‡		†
URINARY																	
BREED	*reference*		‡					‡		-		‡	†	-		‡	
	cafeteria		‡	†			‡	†	‡	-		†	‡	-	‡	†	
DIET	lean				†					-				-	†		†
	obese			†	†		‡	‡	†	-		‡	‡	-		†	‡
ACCRUED																	
BREED	reference	†	‡	‡	‡	‡	‡	‡	‡	‡		‡	‡	†		†	†
	cafeteria	‡	†	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡
DIET	lean	†	‡	‡	‡	‡	‡	‡	‡	‡		†	‡	†	‡	†	‡
	obese	‡	†	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡
METABOLIZED																	
BREED	reference	‡	‡		‡	†	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡
	cafeteria	‡	‡		†			‡			†		†		†	†	†
DIET	lean	†				‡	†	†		‡		‡		‡	†	‡	†
	obese	‡	‡		‡	‡	‡	†	‡	‡	‡	†	‡	†	†		‡

The symbol † indicates a statistically significant ($P<0.05$) difference; the symbol ‡ indicates a higher degree of statistical significance ($P<0.001$) for the differences. The data marked - have not been used in the calculations, since the corresponding levels were zero.


PERCENT AMINO ACIDS
25
20
15
10
5
0
GLX
ASX
ALA
SER
GLY
PRO
HYP
ARG
TYR
PHE
VAL
LEU
ILE
THR
LYS
HIS


LEAN
OBESE
AMINO ACID INGESTED
NOT ABSORBED (%)
0
5
10
15
20
25
30
35
40
45
50
55
GLX ASX ALA SER GLY PRO HYP ARG TYR PHE VAL LEU ILE THR LYS HIS


LEAN RATS
REFERENCE DIET
LEAN RATS
CAFETERIA DIET
OBESE RATS
REFERENCE DIET
OBESE RATS
CAFETERIA DIET
AMINO ACID CONTENT (mmoles)
AMINO ACID CONTENT (mmoles)
0
20
40
60
80
GLX ASX ALA SER GLY PRO HYP ARG TYR PHE VAL LEU ILE THR LYS HIS
GLX ASX ALA SER GLY PRO HYP ARG TYR PHE VAL LEU ILE THR LYS HIS


LEAN RATS
REFERENCE DIET
LEAN RATS
CAFETERIA DIET
OBESE RATS
REFERENCE DIET
OBESE RATS
CAFETERIA DIET
AMINO ACIDS (mmoles)
AMINO ACIDS (mmoles)
0
40
80
120
160
200
GLX ASX ALA SER GLY PRO HYP ARG TYR PHE VAL LEU ILE THR LYS HIS
GLX ASX ALA SER GLY PRO HYP ARG TYR PHE VAL LEU ILE THR LYS HIS

Vol. 25, No. 5, December 1991 BIOCHEMISTRY INTERNATIONAL
Pages 797-806

PLASMA AMINO ACIDS OF LEAN AND OBESE ZUCKER RATS SUBJECTED TO A CAFETERIA DIET AFTER WEANING

Immaculada Rafecas, Montserrat Esteve, Xavier Remesar and Martà Alemany

Departament de Bioquímica i Fisiologia, Universitat de Barcelona
08071 Barcelona, Spain

Received October 29, 1991

Summary

Plasma amino acids of Zucker obese (fa/fa) and lean (Fa/?) rats fed either a reference nonpurified pellet or a cafeteria diet have been studied from 30 to 60 days after birth. Obese rats showed higher plasma branched chain amino acid levels but similar total amino acids, urea and glucose concentrations. The ingestion of a cafeteria diet induced higher levels in many amino acids, as well as in the composite figure in lean rats, but failed to alter total 2-amino nitrogen concentrations in obese rats, despite high levels in several non-essential amino acids and lower values in essential amino acids; urea levels were much lower in rats fed the cafeteria diet. The results are consistent with an impairment of amino acid nitrogen elimination via urea cycle in cafeteria diet-fed rats. This is independent of the hyperinsulinemia-driven plasma accumulation of several essential amino acids induced by genetic obesity. The effects were, then additive.

KEY WORDS: *amino acids; obesity; Zucker fa/fa rat; cafeteria diet*

Introduction

Zucker 'fatty' (*fa/fa*) rats develop obesity shortly after birth (1,2). They are unable to show an adaptive thermogenic response to food (2,3) or cold (2,4), and accumulate large fat reserves (5). The lean counterparts (*Fa/?*) are considered to possess a fully functional thermogenic system, since their responses to cold and diet are normal (6). The Zucker *fa/fa* rat has been extensively used as a model of obesity (7), since the *fa/fa* rats retain more energy and produce a much lower heat output than lean rats (8,9). However, neither the nitrogen metabolism nor the interactions between obesity and nitrogen metabolism of these animals has been extensively studied (10).

The administration of palatable self-selecting diets known as 'cafeteria' diets is another available model of obesity (11). Cafeteria diets have been formulated in a wide range of compositions and administration patterns. Nevertheless they produce very similar results upon the rat's intake: an increase

0158-5231/91/170797-10$01.00/0

in energy (essentially fat), with generalized body weight increases (12,13). The nutrient composition of the ingested diet is fairly constant (13), in spite of a highly variable selection of food.

The rats subjected to a cafeteria diet show a higher carcass nitrogen retention (14), and lower urea cycle enzyme activities (15). The overall result is a higher efficiency of dietary protein nitrogen utilization, since amino acids are used in lower proportion as energy fuels (16), and the availability of essential amino acids for protein synthesis is thus enhanced (17).

The main effect of the cafeteria diets with respect to energy balance is an increase in heat output (13,18), as a consequence of the activation of diet-induced thermogenesis (17), which helps to eliminate the excess energy ingested (19). Both models of obesity have been studied in the same subjects in order to obtain information on their effect on the internal amino acid homeostasis under obesity.

Materials and Methods

Female Zucker rats (bred in the University of Barcelona Animal Facilities from Harlan Olac [Oxon, UK] stock) weaned on day 22, 30 days old and weighing 65-67 g (*Fa/?*) or 80-87 g (*fa/fa*) were used. The animals were housed under standard conditions (21-22 °C, 70-80 % relative humidity, lights on from 08.00 to 20.00). They were fed either standard chow (pelleted non-purified diet type A04 from Panlab, Barcelona, Spain) [containing 592 g/kg metabolizable carbohydrate, 174 g/kg protein, 25 g/kg lipid, 38 g/kg fiber, 56 g/kg minerals and an energy equivalence of 14.2 MJ/kg], or a simplified version of our cafeteria diet (13), consisting of daily fresh offerings of excess chow pellets, liver pâté on cookies, bacon, banana and milk enriched (10 g/L) with a protein, vitamin and mineral supplement (Gevral, Cyanamid Ibérica, Barcelona) and 333 g/L sucrose. The animals had free access to tap water.

Five groups of animals for each strain (*Fa/?* and *fa/fa*) were studied, the first group was killed on day 30 after birth, then two groups were fed the standard chow pellet diet for 15 or 30 days, and killed on days 45 or 60 after birth. The remaining two groups were fed the cafeteria diet and were also killed on days 45 or 60.

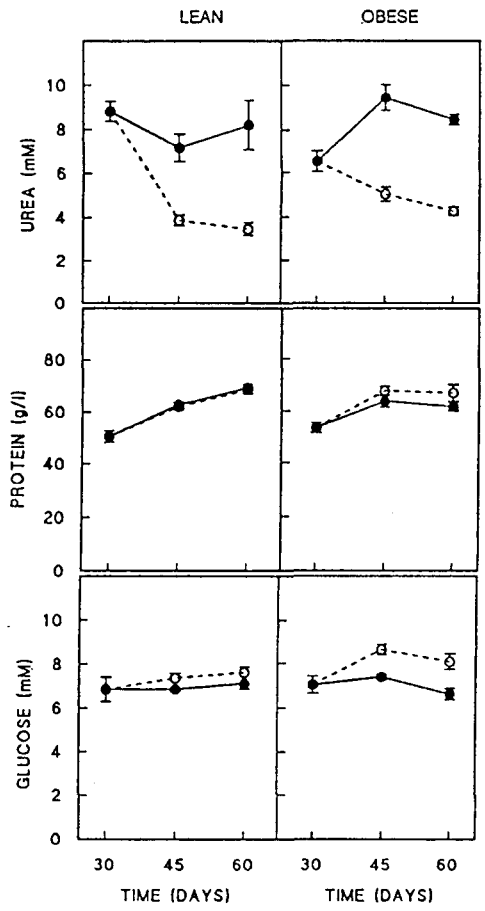
The rats were killed by decapitation. Immediately, samples of blood were recovered and centrifuged. Plasma proteins (20), glucose (21) and urea (22) were determined with standard methods. Aliquots of plasma were deproteinized with 100 g/L trifluoroacetic acid; the acid was removed by evaporation and the precipitates by centrifugation. The supernatants were further ultrafiltered and then individual amino acids were determined with an amino acid analyzer (LKB-Alpha-plus, LKB, Sweden) using a standard o-phtalaldialdehyde method (23) for quantification.

Statistical comparisons were performed by using a standard three-way ANOVA program (BMDP 4-V) run on an IBM 3090 computer.

Results

Figure 1 shows the plasmatic urea, total protein and glucose concentrations of lean and obese rats subjected to the reference and cafeteria diets. No differences due to strain were observed in urea concentrations, which were markedly lower in rats receiving the cafeteria diet. Plasma proteins increased with age, with no differences for strain. Cafeteria diet did not affect lean rat plasma protein concentrations but increased those of the fa/fa animals. For glucose, there were no strain or time effects, but in all cafeteria-fed rats glycemia was higher than in rats fed the reference diet.

Figures 2, 3 and 4 show the individual and total amino acid concentrations of lean and obese rats fed reference and cafeteria diets for 30 days after weaning.



	df	F	P
UREA			
STRAIN	1,42	1.73	0.1960
TIME	2,42	6.00	0.0051
LEAN	2,42	12.68	0.0000
OBESE	2,42	1.19	0.3143
DIET	1,42	111.6	0.0000
PROTEINS			
STRAIN	1,45	0.19	0.6620
TIME	2,45	41.61	0.0000
LEAN	2,45	32.77	0.0000
OBESE	2,45	13.92	0.0000
DIET	1,45	21.84	0.0000
GLUCOSE			
STRAIN	1,41	4.08	0.0500
TIME	2,41	2.38	0.1057
DIET	1,41	21.22	0.0000

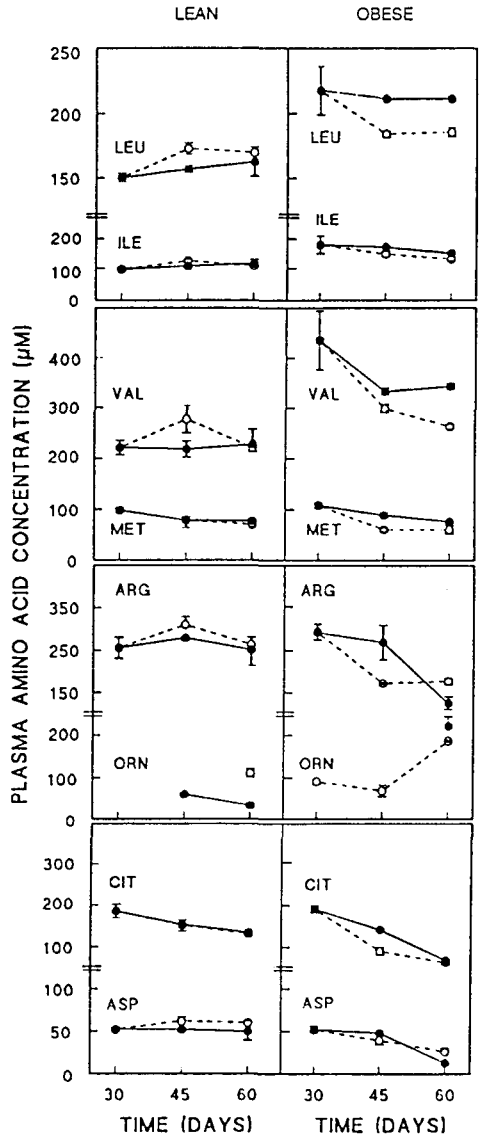
Figure 1

PLASMA UREA, TOTAL PROTEINS AND GLUCOSE CONCENTRATIONS OF LEAN AND OBESE ZUCKER RATS RECEIVING A CONTROL OR CAFETERIA DIET FROM DAYS 30 TO 60 AFTER BIRTH.

Each value represents the mean ± SEM. Black circles and solid line: control (pellet) diet; open circles and dashed line: cafeteria diet. Time represents the age of the animals. On the left are the lean (Fa/?) and on the right the obese (fa/fa) graphs.

Statistical analysis of the differences between groups. A three-way analysis of variance has been carried out using the variables: strain, time and diet. Only the first level of analysis is presented with the degrees of freedom (df), and the F and P values. In the cases where the analysis showed a significant ($P<0.05$) interaction between strain and either time or diet, the second level data have also been presented.

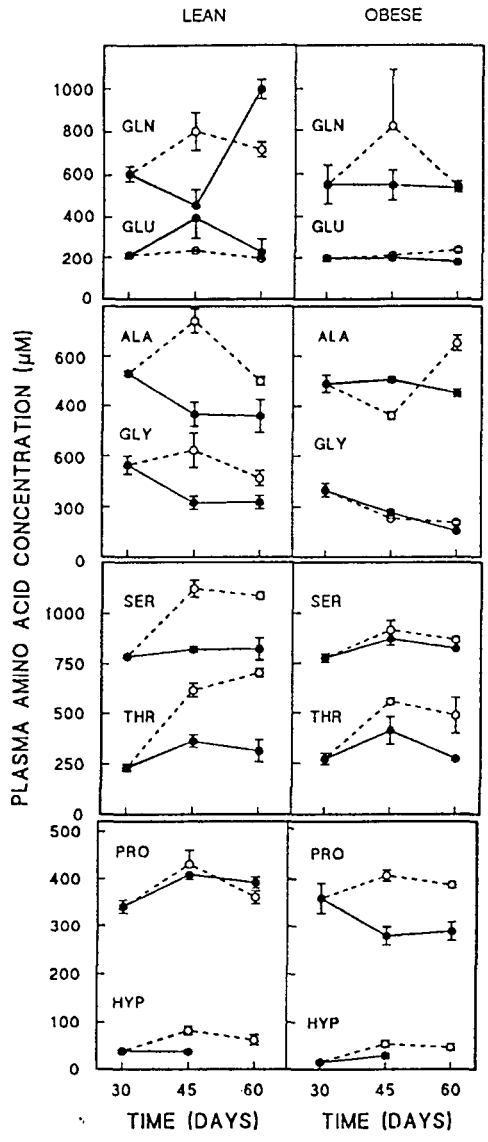
Leucine, isoleucine and valine showed a similar pattern, with higher levels in obese rats. In lean rats, feeding the cafeteria diet resulted in higher (leucine) or unaltered (isoleucine and valine) the reference diet pattern. However, in obese rats, the cafeteria diet induced lower concentrations for all three branched chain amino acids. Methionine showed a similar pattern, but there were no differences between the two strains.



		df	F	P
LEU •				
	STRAIN	1,36	58.70	0.0000
	TIME	2,36	0.04	0 9584
	LEAN	2,36	1.94	0.1580
	OBESE	2,36	2.05	0.1429
	DIET	1,36	1.41	0.2433
	LEAN	1,36	5.61	0.0234
	OBESE	1,36	11.42	0.0018
ILE				
	STRAIN	1,38	24.64	0.0000
	TIME	2,38	0.80	0.4579
	DIET	1,38	0.96	0.3342
	LEAN	1,38	0.59	0.4470
	OBESE	1,38	4.07	0.0508
VAL				
	STRAIN	1,38	34.91	0.0000
	TIME	2,38	3.66	0.0353
	LEAN	2,38	0.55	0.5828
	OBESE	2,38	7.76	0.0015
	DIET	1,38	1.85	0.1822
	LEAN	1,38	1.44	0.2380
	OBESE	1,38	7.11	0.0112
MET				
	STRAIN	1,41	0.08	0.7753
	TIME	2,41	13.65	0.0000
	DIET	1,41	16.19	0.0002
ARG				
	STRAIN	1,36	18.56	0.0001
	TIME	2,36	8.86	0.0007
	LEAN	1,36	2.58	0.0899
	OBESE	1,36	13.00	0.0001
	DIET	1,36	0.44	0.5119
	LEAN	1,36	2.94	0.0948
	OBESE	1,36	4.54	0.0400
ORN				
	STRAIN	1,12	29.23	0.0002
	TIME	2,12	15.42	0.0005
	LEAN	1,12	0.33	0.5782
	OBESE	1,12	34.43	0.0000
	DIET	1,12	2.44	0.1442
	LEAN	1,12	8.18	0.0146
	OBESE	1,12	2.41	0.1464
CIT				
	STRAIN	1,30	26.25	0.0000
	TIME	2,30	56.89	0.0000
	LEAN	2,30	10.18	0.0004
	OBESE	2,30	56.79	0.0000
	DIET	1,30	19.40	0.0001
	LEAN	1,30	2.64	0.1146
	OBESE	1,30	18.60	0.0002
ASP				
	STRAIN	1,35	51.02	0.0000
	TIME	2,35	11.79	0.0001
	LEAN	2,35	0.48	0.6217
	OBESE	2,35	24.70	0.0000
	DIET	1,35	0.43	0.5183
	LEAN	1,35	6.36	0.0164
	OBESE	1,35	2.44	0.1270

Figure 2
PLASMA LEUCINE, ISOLEUCINE, VALINE, METHIONINE, ARGININE, ORNITHINE, CITRULLINE AND ASPARTATE CONCENTRATIONS OF LEAN AND OBESE ZUCKER RATS RECEIVING A CONTROL OR CAFETERIA DIET FROM DAYS 30 TO 60 AFTER BIRTH.
The conventions used are the same as in table 1.

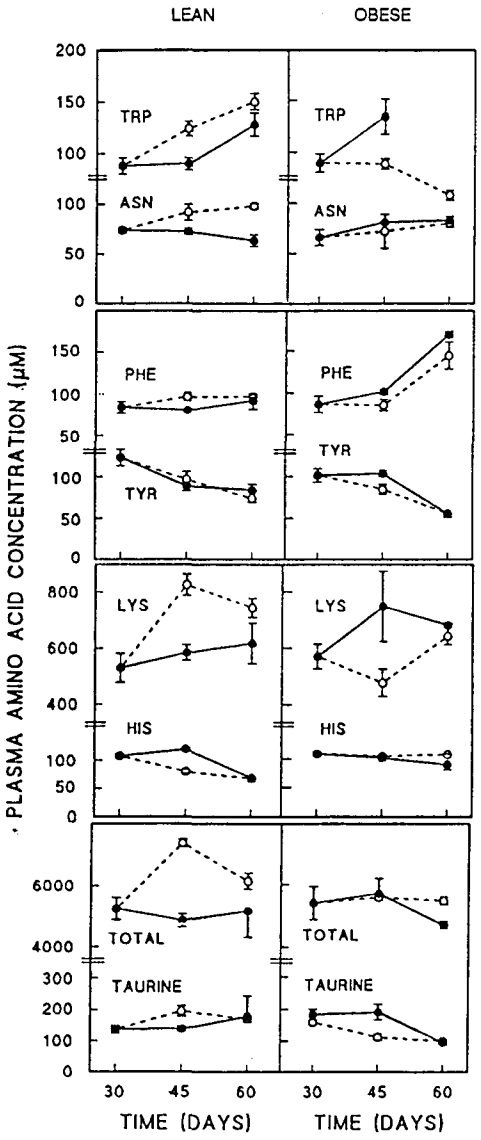
Arginine levels did not change with age in lean but decreased significantly in elder obese rats. Similarly, diet affected arginine levels in obese but did not in lean rats. There were also significant strain differences for ornithine and citrulline levels. Obese rats showed a more marked decrease in citrulline levels with age than their lean counterparts.



		df	F	P
GLN				
	STRAIN	1,38	4.97	0.0318
	TIME	2,38	1.32	0.2783
	LEAN	2,38	1.14	0.3315
	OBESE	2,38	3.68	0.0346
	DIET	1,38	2.84	0.0999
GLU				
	STRAIN	1,37	2.66	0.1115
	TIME	2,37	1.37	0.2661
	DIET	1,37	0.24	0.6261
ALA				
	STRAIN	1,37	1.12	0.2973
	TIME	2,37	0.07	0.9354
	LEAN	2,37	6.85	0.0029
	OBESE	2,37	4.58	0.0167
	DIET	1,37	15.68	0.0003
	LEAN	1,37	27.19	0.0000
	OBESE	1,37	0.42	0.5233
GLY				
	STRAIN	1,32	35.55	0.0000
	TIME	2,32	9.00	0.0008
	DIET	1,32	1.80	0.1892
	LEAN	1,32	10.71	0.0026
	OBESE	1,32	0.99	0.3266
SER				
	STRAIN	1,34	24.95	0.0000
	TIME	2,34	10.60	0.0000
	LEAN	2,34	11.30	0.0002
	OBESE	2,34	1.78	0.1842
	DIET	1,34	90.05	0.0000
	LEAN	1,34	99.79	0.0000
	OBESE	1,34	14.35	0.0006
THR				
	STRAIN	1,36	2.80	0.1031
	TIME	2,36	28.61	0.0000
	LEAN	2,36	18.97	0.0000
	OBESE	2,36	11.77	0.0001
	DIET	1,36	131.5	0.0000
	LEAN	1,36	118.0	0.0000
	OBESE	1,36	31.30	0.0000
PRO				
	STRAIN	1,36	3.31	0.0771
	TIME	2,36	5.48	0.0084
	LEAN	2,36	9.66	0.0004
	OBESE	2,36	0.77	0.4691
	DIET	1,36	24.36	0.0000
HYP				
	STRAIN	1,17	13.31	0.0020
	TIME	2,17	11.92	0.0006
	DIET	1,17	29.62	0.0000

Figure 3
PLASMA GLUTAMINE, GLUTAMATE, ALANINE, GLYCINE, SERINE, THREONINE, PROLINE AND HYDROXYPROLINE CONCENTRATIONS OF LEAN AND OBESE ZUCKER RATS RECEIVING A CONTROL OR CAFETERIA DIET FROM DAYS 30 TO 60 AFTER BIRTH.
The conventions used are the same as in table 1

Aspartate concentrations again showed no change in lean but noticeable decreases in obese rats with time; cafeteria diet did not affect obese but increased lean rat aspartate levels. Glutamine showed dramatic variations during the period studied in lean rats. Obese rats showed higher and more stable levels over time. No significant effects of the diet were observed because of a wide dispersion of data. Glutamic acid levels did not show any significant effects of time, diet or strain.



		df	F	P
TRP				
	STRAIN	1,30	8.04	0.0081
	TIME	2,30	4.00	0.0289
	LEAN	2,30	13.17	0.0001
	OBESE	2,30	3.95	0.0300
	DIET	1,30	0.02	0.8870
	LEAN	1,30	17.90	0.0002
	OBESE	1,30	8.34	0.0071
ASN				
	STRAIN	1,37	0.45	0.5042
	TIME	2,37	2.74	0.0773
	DIET	1,37	8.69	0.0055
	LEAN	1,37	20.35	0.0001
	OBESE	1,37	0.01	0.9293
PHE				
	STRAIN	1,35	24.49	0.0000
	TIME	2,35	27.17	0.0000
	LEAN	2,35	0.73	0.4912
	OBESE	2,35	30.68	0.0000
	DIET	1,35	0.29	0.5938
TYR				
	STRAIN	1,39	7.88	0.0078
	TIME	2,39	35.15	0.0000
	DIET	1,39	10.99	0.0020
LYS				
	STRAIN	1,37	1.13	0.2955
	TIME	2,37	3.78	0.0321
	DIET	1,37	2.24	0.1434
	LEAN	1,37	21.27	0.0000
	OBESE	1,37	4.05	0.0515
HIS				
	STRAIN	1,39	7.22	0.0105
	TIME	2,39	6.00	0.0054
	DIET	1,39	2.36	0.1326
	LEAN	1,39	0.46	0.4995
	OBESE	1,39	7.24	0.0105
TOTAL AMINO ACIDS				
	STRAIN	1,32	1.50	0.2274
	TIME	2,32	1.48	0.2401
	DIET	1,32	10.20	0.0027
	LEAN	1,32	17.18	0.0002
	OBESE	1,32	0.34	0.5630
TAURINE				
	STRAIN	1,34	6.38	0.0164
	TIME	2,34	0.41	0.6701
	LEAN	2,34	2.01	0.1497
	OBESE	2,34	3.81	0.0322
	DIET	1,34	0.52	0.4758
	LEAN	1,34	0.89	0.3517
	OBESE	1,34	4.03	0.0528

Figure 4

PLASMA TRYPTOPHAN, ASPARAGINE, PHENYLALANINE, TYROSINE, LYSINE, HISTIDINE, TAURINE AND COMBINED TOTAL AMINO ACID CONCENTRATIONS OF LEAN AND OBESE ZUCKER RATS RECEIVING A CONTROL OR CAFETERIA DIET FROM DAYS 30 TO 60 AFTER BIRTH.

The conventions used are the same as in table 1.

Alanine concentrations showed a different pattern of variation with time for lean and obese rats. The cafeteria diet induced much higher alanine levels in lean and no significant overall effects (but a different pattern) in fa/fa rats. Glycine showed a similar pattern to that of alanine for pellet-fed rats. In all cases there was a decrease in glycine concentrations with time; the diet had no effect on the pattern for

obese rats. Serine and threonine presented a similar pattern, with higher concentrations in cafeteria-fed than in reference diet–fed rats. There were significant strain differences for serine but not for threonine.

The administration of the cafeteria diet did not alter the proline levels in lean but increased them considerably in obese rats. Hydroxyproline levels were lower in obese than in lean rats; in both cases cafeteria diet induced higher circulating levels.

The plasma concentrations of tryptophan of lean rats increased with time, those of cafeteria-fed rats being raised more than those of reference diet–fed rats. Chow diet-fed obese rats showed higher tryptophan levels than those fed cafeteria diet. The only changes observed in asparagine levels were higher concentrations induced by cafeteria diet in lean rats.

Phenylalanine levels did not change with time in lean but increased considerably in obese rats. Tyrosine concentrations showed a uniform trend towards lower values with time in both strains, cafeteria diet inducing lower concentrations in obese rats.

Lysine increased with time in rats fed the standard chow diet; cafeteria feeding resulted in higher levels for lean and lower for obese rats. Histidine decreased with age, the differences between strains and diets being also significant.

Taurine concentrations did not change with time in lean but decreased in obese rats. Cafeteria diet did not affect the animals fed the reference diet but induced lower concentrations in obese rats.

The sum of all amino acids showed a remarkable uniformity both with respect to time and strain; the diet, however, induced some differences, with higher combined concentrations in lean rats that received cafeteria diet, and no significant effects for obese rats.

Discussion

In obesity, the relative excess of energy available (24), helps preserve the protein energy, since there is no immediate need to oxidize protein as energy fuel. This protein–sparing effect is clearly apparent in the nitrogen balances of rats fed a fat–rich cafeteria diet (25). Since the need for protein is practically limited to the sustainment of growth and turnover, the rat chooses a lower proportion of protein in its diet, but its protein intake is comparable to that of a standard diet (13). The nitrogen management of cafeteria-fed rats is more efficient in general terms than that of rats fed control diets (15). Despite a comparable nitrogen intake (13), the adult cafeteria-fed rats manage to retain a amount of protein comparable with that of the rats fed control or reference diets (26), thanks to the higher digestibility of the cafeteria diet (27), a more efficient intestinal uptake, lower nitrogen excretion (15,25) and more efficient retention in the body (19,28).

Most essential amino acids showed a very different effect of cafeteria-feeding upon the pattern of change in plasma concentrations with age. In general, the cafeteria diet raised the concentrations in lean and decreased them in obese rats. In obese rats, the cafeteria diet caused increases only in a few amino acids, namely serine, threonine, proline and hydroxyproline. These increases agree with those described in the literature (29), with little variation in other amino acids (30). Cafeteria feeding can induce hyperinsulinemia, and eventually insulin resistance (31). This results in lower oxidation of leucine and other branched chain amino acids (32); the outcome is a tendency towards the conservation of branched chain amino acids and a relative accumulation of leucine in plasma (30) as partially observed here.

Hypothalamic obesity is known to alter nitrogen metabolism in rats by increasing tissue protein catabolism or diminishing its synthesis (33). The appetite for protein is maintained intact in this obesity, as it is with genetic obesity (34). In contrast with cafeteria diet–fed rats, hypothalamic cut–provoked obesity is characterized by high urea production (35) as a consequence of increased amino acid oxidation, partly driven by higher glucagon (35). Genetically obese rodents have an increased ability to incorporate amino acid hydrocarbon skeletons into lipids (36), as well as normal urea levels/production (37). In contrast, these animals show an impaired protein turnover (38), probably another consequence of their essential hyperinsulinemia (39). The consequences are easily seen in the plasma aminogram: many essential amino acids were found in higher concentrations than in lean controls, in contrast with the lack of changes observed in old rats (40). Branched chain amino acids and their 2-ketoacids show higher concentrations in obese humans (41,42), because insulin resistance lowers their metabolism (43).

Since in obesity the synthesis of protein is maintained in spite of a lowered turnover (38), and part of the amino acid energy is driven towards lipid synthesis, the outcome is a similar total amino acid figure for lean and obese rats fed a standard diet.

The differences between amino acid levels of lean and obese rats fed cafeteria diet affect the majority of amino acids, with consistently lower values in most of the essential amino acids, namely branched chain amino acids, whose hydrocarbon skeletons are commonly used for lipid synthesis.

The effect of cafeteria-feeding upon lean and obese rats is very different, since the nitrogen retention of cafeteria-fed rats is not due solely to hyperinsulinemia (44), but more probably to hyperphagia (29). The effects of lowering urea production and increasing amino acid availability for protein synthesis are fully patent in both groups, with a different overall behavior: lean rats accumulate more amino acids in plasma because they are not using them for energy and their capability to synthesize proteins is limited. Cafeteria-fed obese Zucker rats, on the other hand, retain some capability to incorporate amino acids into lipid, but they have also blocked the production of urea. The result is a lower increase in circulating amino acids when compared with pellet-fed controls, and, very probably, the elimination of the excess nitrogen through mechanisms other than urea (45). Plausibly, the lower ability of the obese rat kidney to retain nutrients (46) and, perhaps, higher rates of elimination of other nitrogen forms could eventually account for this difference.

The divergent behavior of the time course of change in phenylalanine and tyrosine levels in obese rats, as compared with their lean counterparts may suggest an alteration of the ability of the rat to convert phenylalanine into tyrosine. The effect of diet seems to be minor in that respect. This is so because phenylalanine doubles its levels in one month and tyrosine just halves. Since the content of both amino acids in the diets is the same as in lean controls, this change may have some bearing (right now unexplored) on the metabolic changes suffered by the fa/fa rat in later life.

The overall conclusion that can be drawn from this study is the remarkable maintenance of plasma 2-amino nitrogen concentration despite the administration of a diet with high quality protein under conditions of hyperphagia as well as under conditions of genetic obesity. The effects of cafeteria diet upon nitrogen disposal mechanisms, very different from the active oxidation of amino acids found in genetic obesity, combine with the latter to offer a picture in which both strategies prevail at the same time, resulting in the maintenance of circulating 2-amino nitrogen levels.

Acknowledgements

This study was supported by grants nos. PB86-0512 and PB88-0208 from the 'Dirección General de Investigación Científica y Técnica' from the Government of Spain. Thanks are given to Robin Rycroft for his help in the correction of the manuscript.

References

1. Boulangé, A., Planche, E. and de Gasquet, P. (1979) *J. Lipid Res.* 20: 857-864.
2. Bray, G.A. and York, D.A. (1979) *Physiol. Rev.* 59: 719-809.
3. Holt, S.J., York, D.A. and Fitzsimons, J.T.R. (1983) *Biochem. J.* 214: 215-223.
4. Triandafillou, J. and Himms-Hagen, J. (1983) *Am. J. Physiol.* 224: E145-E150.
5. Levin, B.E., Triscari, J. and Sullivan, A.C. (1980) *Pharmacol. Biochem. Behav.* 13: 107-113.
6. Kaul, R., Schmidt, I and Carlisle, H. (1985) *Int. J. Obesity* 9: 401-409.
7. Kurtz, T.W., Morris, R.C. and Pershadsingil, H.A. (1989) *Hypertension* 13: 896-901.
8. Diersen-Schade, D.A., Sershen, D.J. and Cleary, M.P. (1988) *Nutr. Res.* 8: 1029-1039.
9. Rafecas, I., Domènech, T., Esteve, M., Remesar, X., Argilés, J.M. and Alemany, M. (1989) *Nutr. Res.* 9: 1407-1413.
10. Harris, R.B.S., Tobin, G. and Hervey, G.R. (1988) *J. Nutr.* 118: 503-514.
11. Sclafani, A.A. and Springer, D. (1976) *Physiol. Behav.* 17: 461-471.
12. Naim, M., Brand, J., Kare, M.R. and Carpenter, R.G. (1985) *J. Nutr.* 115: 1447-1458.
13. Prats, E., Monfar, M., Castellà, J., Iglesias, R. and Alemany, M. (1989) *Physiol. Behav.* 45: 263-272.
14. Barr, H.G. and McCracken, K.J. (1984) *Br. J. Nutr.* 51: 279-387.
15. Barber, T., Viña, J.R., Viña, J. and Cabo, J. (1985) *Biochem. J.* 230: 675-681.
16. Henry, C.J.K., Rivers, J.P.W. and Payne, P.P. (1986) *Nutr. Clin. Nutr.* 4: 87-92.
17. Rothwell, N.J. and Stock, M.J. (1982) In: *Brown adipose tissue* (Trayhurn, P. and Nicholls, D., eds.), pp. 269-298, Edward Arnold, London.
18. Castellà, J. and Alemany, M. (1986) *Comp. Biochem. Physiol. A* 85: 203-206.
19. Rothwell, N.J. and Stock, M.J. (1982) *J. Physiol.* 328: 371-377.
20. Lowry, O.H., Rosebrough, M.J., Farr, A.L. and Randall, R.J. (1951) *J. Biol. Chem.* 193: 265-275.
21. Trinder, P. (1969) *Ann. Clin. Biochem.* 6: 24-27.
22. Fawcett, J.K. and Scott, J.E. (1960) *J. Clin. Pathol.* 12: 156-163.
23. Benson, J.R. and Hare, P.E. (1975) *proc. Nat. Acad. Sci. USA* 72: 619-622.
24. Bray, G.A. and York, D.A. (1972) *Am. J. Physiol.* 223: 176-179.
25. Henry, C.J.K., Rivers, J.P.W. and Payne, P.R. (1987) *Nutr. Res.* 7: 1243-1252.
26. Gianotti, M., Roca, P. and Palou, A. (1988) *Horm. Metabol. Res.* 20: 208-212.
27. Rothwell, N.J. and Stock, M.J. (1981) *Metabolism* 30: 673-678.
28. Iglesias, R., Andrés, V., Castellà, J. and Alemany, M. (1986) *Nutr. Rep. Int.* 34: 229-239.
29. Calles-Escandon, J., Cunningham. J. and Felig, P. (1984) *Metabolism* 33: 364-368.
30. Gianotti, M., Roca, P. and Palou, A. (1990) *Arch. Internat. Physiol. Biochim.* 98: 155-161.
31. Cunningham, J., Calles, J., Eiskowitz, L., Zawalich, W. and Felig, P. (1983) *Diabetes* 32: 1023-1027.
32. Marchesini, G., Cassarani, S., Checchia, A., Bianchi, G., Bua, V., Zoli, M. and Pisi, E. (1987) *Metabolism* 36: 1096-1100.
33. Holm, H., Hustvedt, B.E. and Løbø, A. (1973) *Metabolism* 22: 1377-1387.
34. Anderson, G.H., Leprohon, C., Chambers, J.W. and Coscina, D.V. (1979) *Physiol. Behav.* 23: 751-755.
35. Karakash, C., Rohner-Jeanrenaud, F., Hustvedt, B.E. and Jeanrenaud, R.B. (1980) *Am. J. Physiol.* 238, E32-E37.
36. Dunn, M.A. and Hartsook, E.W. (1980) *J. Nutr.* 110: 1865-1879.
37. Schirardin, H., Bach, A., Schaeffer, A., Bauer, M. and Werhya, A. (1979) *Arch. Int. Physiol. Biochim.* 87: 275-289.
38. Chan, C.P., Hausen, R.J. and Stern, J.S. (1985) *J. Nutr.* 115: 959-969.
39. Turkenkopf, I.J., Johnson, P.R. and Greenwood, R.C. (1982) *Am. J. Physiol.* 242: E220-E225.

40. Tsuda, T.T., Ohkubo, T., Kamiguchi, H., Tsuda, M., Katsunuma, T. and Tamamura, M. (1989) *J. Nutr.* 119: 1327-1332.
41. Felig, P., Wahren, J., Hendler, R. and Brundin, T. (1974) *J. Clin. Invest.* 53: 582-590.
42. Schauder, P., Zavelberg, D., Langer, K. and Herbertz, L. (1987) *Am. J. Clin. Nutr.* 46: 58-60.
43. Forlani, G., Vannini, P., Marchesini, G., Zoli, M., Ciavarella, A. and Pisi, E. (1984) *Metabolism* 33: 147-150.
44. Barber, T., Estornell, E., Esteller, R., Gómez,D. and Cabo, J. (1987) *Mol. Cell. Endocrinol.* 50: 15-22.
45. Costa, G., Ullrich, L., Ferenc, K. and Holland, J.F. (1968) *Nature* 218: 546-551.
46. Fiske, W.D., Blouin, R.D., Mitchell, B. and McNamara, P.J. (1986) *Int. J. Obesity* 10: 175-183.

treball enviat per publicar a BIOCHEMISTRY INTERNATIONAL

Urinary loss of amino acids in obese Zucker rats

Montserrat Esteve, Immaculada Rafecas, José Antonio Fernández-López, Xavier Remesar and Marià Alemany

Departament de Bioquímica i Fisiologia, Universitat de Barcelona, 08028 Barcelona Spain.

Summary

The urine free amino acid concentration of 30-60 day Zucker lean and obese rats fed during one month reference or cafeteria diets have been measured. Essential amino acids were found in very low concentrations in urine, the only levels not negligible were those of threonine, lysine, and specially histidine. The data presented confirm the effect of diet on the pattern and magnitude of urinary amino acids. The highest losses are found in animals receiving an energy-rich cafeteria diet. The urinary losses of dietary amino acids were a —low— fraction of one per cent for most amino acids, being higher only for alanine and glycine and highest for hydroxyproline.

The urine concentration of glycine was comparable or higher than that of plasma and was not affected by diet or obesity. Alanine levels ranged from less than 10 % of the plasma levels to 2.25–fold, being higher in cafeteria-fed rats and in obese animals. The relative uniformity of the volume of urine formed every day contrasts with the large differences in urea concentration, a major osmotic component. Lean rats concentrated plasma about 120 to 130-fold when fed the reference and 180-190 the cafeteria diet. The corresponding figures for obese rats were: 160-170 for reference and 150-180 for cafeteria diet.

KEY WORDS: *urine amino acids; excretion; urea; renal transport*

Introduction

The main N-containing component in rat and human urine is urea, end-product of ammonia detoxification and amino acid catabolism. Urine also contains small and variable amounts of other nitrogen compounds, such as urate, creatinine and ammonia (1). The kidney is able to retain most of the valuable plasma solutes thanks to its selective reabsorption: glucose and most amino acids (2). However, this efficiency may be affected by physiological state in which amino acid transport is affected (3,4).

As a consequence of the efficiency of amino acid absorption along the renal tubules, the amount of amino acid present in urine is usually negligible for most species (5), a condition directly related with the ability to retain and preserve amino acid nitrogen. The synthesis of urea, and the overall urinary elimination of N compounds depends essentially on the proportion of protein in the diet

(6). The rat is singular because excretes a tiny, albeit significant, amount of 2-amino nitrogen in urine (7), especially during early postnatal development (8). In the adult, however, a large proportion of urine amino acids is made up by imino acid peptides, mainly hydroxyproline, as well as other non-recyclable amino acids as methyl-histidine (9).

Obesity affects plasma amino acid levels (10), extra-urinary losses of nitrogen (11) and protein assimilation (12). The latter may be partly due to obesity-induced alterations on the intestine and liver amino acid transport systems (13). Since the type of obesity modifies amino acid management in the form of excretion of excess N (and its proportions) and in the mechanisms of tissue uptake —ultimately on amino acid transport—, and both are directly implicated in the control of the presence of amino acids in urine, we have studied the effect of diet on the loss of amino acids through urine in Zucker obese rats.

Materials and Methods

The animals studied were obese (fa/fa) or lean (Fa/?) female Zucker rats, bred at the University of Barcelona Animal Service from Charles River France heterozygous parents; they were used 30 days after birth (weaned on day 22). The rats were housed in polycarbonate metabolic cages (Tecniplast Gazzada, Italy) which allowed the daily estimation of the consumption of individual food items. The cages were maintained in a light (on from 08.00 to 20.00), humidity (70-80 % relative humidity) and temperature (21-22 °C) controlled environment. Series of both lean and obese rats were fed a commercial reference pellet diet (type A04 from Panlab, Barcelona, containing 170 $g \cdot kg^{-1}$ protein, 587 $g \cdot kg^{-1}$ digestible carbohydrate, and 30 $g \cdot kg^{-1}$ lipids) and tap water *ad libitum*. The cafeteria diet (14) was changed daily and was presented in excess.

For each breed (lean or obese) and dietary group (reference– or cafeteria–fed) three sets (5-7 each) of animals were studied, being killed at 30 (0 days of dietary treatment), 45 (15 days of treatment) or 60 (30 days of treatment) days after birth. The 60–day group (kept in metabolic cages) was used for daily individual measurement of balances (11). The other rats (45–day, and 30–day old) were kept individually in ordinary cages, where they were fed the same diets. On days 30, 45 or 60, the allotted groups of rats were killed by decapitation, their blood was used for the estimation of plasma urea (15), total 2-amino nitrogen (16) and individual amino acids (17).

Urine samples were cleaned by ultrafiltration, and their amino acid phenyl-hydantoines were prepared (17). All series of determinations included standards of known amino acid mixtures. Prior to derivatization, an internal standard of norleucine was also added to each sample; other series of standards were used for correction of the efficiency of derivatization and losses of amino acids due to the formation of adducts. The amino acid analyses were carried out in an LKB 2150 high performance liquid chromatograph using Spherisorb ODS-2 (5 μm) columns (150x4 mm) and a gradient of ammonium acetate/acetonitrile/methanol as mobile phase at 60 °C (17). Due to methodological constraints, no reliable data for Trp, Cys and Met were obtained, thus they are not presented. The content of nitrogen in the urine samples was measured with a Carlo Erba NA-1500 elemental analyzer.

The plasma amino acid levels of 30 and 45-day rats were computed so as to obtain a representative 'mean' of the 30-45 day period; the same was done with the 45 and 60-day rats. These figures were compared with the corresponding 15-day–pooled urine amino acids in order to obtain the ratios presented in Figure 1.

Statistical comparisons between the groups were made with a standard two– or three–way ANOVA program, as well as with the Student's *t* test.

Results

Table 1 shows the urine free amino acid concentrations of Zucker lean and obese rats. There was a marked overall effect of age on the mean amino acid concentrations: in general, the concentrations for the second period —days 45 to 60— were higher than for the first —days 30 to

Table 1

Urine amino acid concentrations of Zucker lean and obese rats fed reference and cafeteria diets.

amino acid	age group	lean reference	lean cafeteria	obese reference	obese cafeteria	statistical comparisons breed	diet	time
Glx	30-45	56.8 ± 6.8	69.7 ± 6.5	70.2 ± 4.8	62.5 ± 9.4			$T/T_O/T_R$
	45-60	*90.7 ± 11.5*	*70.5 ± 5.6*	*98.2 ± 5.4*	*93.7 ± 7.7*			
Asx	30-45	46.3 ± 4.8	67.0 ± 4.6	78.8 ± 4.7	73.8 ± 11.1	$B/B_R/B_C$		$T/T_O/T_R$
	45-60	*63.8 ± 4.3*	*57.0 ± 3.7*	*110.3 ± 7.7*	*118.2 ± 9.6*			
Ala	30-45	37.7 ± 2.7	29.8 ± 1.9	137.3 ± 14.9	206.3 ± 58.4	$B/B_R/B_C$	$D/D_L/D_O$	$T/T_L/T_O/T_R/T_C$
	45-60	*118.0 ± 34.4*	*219.5 ± 25.9*	*217.0 ± 64.1*	*905.8 ± 289.2*			
Ser	30-45	40.0 ± 3.3	39.3 ± 2.1	54.3 ± 3.7	34.1 ± 2.5	B	D/D_O	$T/T_O/T_R$
	45-60	*79.3 ± 13.3*	*41.7 ± 3.4*	*88.5 ± 7.0*	*63.7 ± 7.3*			
Gly	30-45	247.3 ± 39.7	288.0 ± 2.1	227.0 ± 27.5	268.7 ± 31.3			$T/T_L/T_O/T_C$
	45-60	*371.3 ± 75.8*	*367.5 ± 16.1*	*255.1 ± 36.0*	*346.3 ± 19.5*			
Pro	30-45	31.5 ± 2.8	35.7 ± 4.5	49.2 ± 4.7	60.0 ± 13.3	B/B_C	D_O	$T/T_L/T_O/T_C$
	45-60	*59.7 ± 5.2*	*42.2 ± 6.5*	*53.7 ± 5.5*	*126.8 ± 11.0*			
Hyp	30-45	39.5 ± 4.9	58.0 ± 7.7	<25	52.7 ± 11.2	$B/B_R/B_C$	D/D_O	T_R
	45-60	*56.0 ± 7.0*	*57.7 ± 11.9*	*<25*	*114.5 ± 18.6*			
Arg	30-45	8.0 ± 0.5	8.3 ± 0.7	11.0 ± 1.7	14.2 ± 1.9	B/B_C		T_R
	45-60	*13.7 ± 1.4*	*7.0 ± 1.5*	*16.0 ± 1.3*	*26.1 ± 3.8*			
Tyr	30-45	<2	<2	<2	<2	—		
	45-60	*<2*	*<2*	*<2*	*<2*			
Phe	30-45	7.8 ± 1.5	8.2 ± 1.6	7.8 ± 0.8	8.7 ± 1.2			$T/T_O/T_R$
	45-60	*12.2 ± 2.2*	*7.5 ± 0.8*	*15.7 ± 3.6*	*10.8 ± 1.7*			
Val	30-45	5.8 ± 0.9	3.0 ± 0.7	12.0 ± 2.2	<2	B_C	D/D_O	$T/T_L/T_R/T_O$
	45-60	*9.0 ± 0.7*	*7.2 ± 1.5*	*17.5 ± 2.7*	*<2*			
Leu	30-45	3.2 ± 0.5	5.2 ± 0.6	5.0 ± 0.6	<2	B_C	D_O	T_R/T_O
	45-60	*6.2 ± 0.9*	*4.2 ± 0.2*	*8.8 ± 1.4*	*<2*			
Ile	30-45	<2	<2	<2	<2	—		
	45-60	*<2*	*<2*	*<2*	*<2*			
Thr	30-45	99.2 ± 38.1	246.5 ± 20.4	66.5 ± 5.7	39.8 ± 2.5	B	D/D_O	T_O
	45-60	*83.5 ± 11.8*	*39.7 ± 2.6*	*108.0 ± 9.3*	*94.7 ± 12.9*			
Lys	30-45	24.8 ± 2.7	38.3 ± 3.2	57.8 ± 4.7	40.0 ± 5.9	B/B_R	D	T_R
	45-60	*44.2 ± 3.2*	*41.2 ± 4.7*	*83.8 ± 4.3*	*75.7 ± 6.7*			
His	30-45	18.2 ± 1.9	33.2 ± 3.0	20.3 ± 1.3	29.3 ± 3.2		D/D_L	T_O
	45-60	*29.0 ± 1.8*	*42.5 ± 5.0*	*36-3 ± 3.8*	*58.5 ± 5.9*			
Urea ($mmol \cdot l^{-1}$)	30-45	983 ± 83	663 ± 84	1467 ± 28	782 ± 41	$B/B_R/B_C$	$D/D_L/D_O$	T_O
	45-60	*998 ± 42*	*583 ± 65*	*1741 ± 132*	*850 ± 117*			

Amino acid concentrations are expressed in $\mu moles \cdot l^{-1}$, urea levels in $mmoles \cdot l^{-1}$. The data shown are the mean ± SEM of pooled 15-day (30-45 or 45-60 days) urines of 5-6 animals per group.
*Statistical comparisons between the groups (three-way ANOVA): the symbols indicate that there is a significant ($P<0.05$) or highly significant —in **bold**— ($P<0.001$) effect of the given parameter: B = breed, D = diet and T = time; subindexed letters limit the significance to the indicated groups: $_R$ = reference diet, $_C$ = cafeteria diet; $_L$ = lean rats, $_O$ = obese rats; non-subindexed letters refer to the global significance of the effects of breed, diet or time. Only the significant relations are shown. The effects of breed or diet upon individual 15-day time stretches are not shown.*

45—. The levels of most neutral essential amino acids were negligible or very low for all groups studied, whilst those of some neutral non-essential amino acids were considerable. In all groups, the highest levels were for glycine and alanine. Obesity resulted in significant changes for aspartate plus asparagine, alanine, proline, hydroxyproline, arginine and, to a lesser extent, serine, valine, leucine, threonine and lysine. The effects of diet were more marked for alanine, serine, hydroxyproline, valine, threonine and histidine, with less effects on proline, leucine and lysine. Also in general, cafeteria-fed rats showed higher urinary amino acid levels. Obesity resulted in an altered pattern of change with time, with levels higher for some amino acids than the controls fed the reference diet.

Table 2

Energy and nitrogen intake, plasma levels and excretion in Zucker rats fed reference and cafeteria diets for 30 days.

parameter	age group	lean reference	lean cafeteria	obese reference	obese cafeteria	statistical comparisons breed	diet	time
net energy intake ($kJ \cdot day^{-1}$)	30-45	189 ± 9	256 ± 7	305 ± 12	409 ± 13	$B/B_R/B_C$	$D/D_L/D_O$	T/T_R
	45-60	*247 ± 7*	*307 ± 6*	*422 ± 5*	*459 ± 16*			
net N intake ($mmol \cdot day^{-1}$)	30-45	25.14 ± 1.14	23.76 ± 0.67	40.57 ± 1.62	34.05 ± 1.48	$B/B_R/B_C$	$D/D_L/D_O$	T/T_R
	45-60	*32.81 ± 0.90*	*26.90 ± 0.52*	*56.14 ± 0.66*	*37.19 ± 1.52*			
N excreted in urine ($mmol \cdot day^{-1}$)	30-45	9.62 ± 0.29	5.48 ± 0.14	18.62 ± 0.30	9.90 ± 0.71	$B/B_R/B_C$	$D/D_L/D_O$	$T/T_R/T_O$
	45-60	*15.76 ± 0.19*	*8.52 ± 0.10*	*27.76 ± 1.52*	*14.29 ± 1.38*			
urine volume ($ml \cdot day^{-1}$)	30-45	5.1 ± 1.16	3.79 ± 0.42	6.98 ± 0.61	6.62 ± 0.57			$T/T_L/T_O$
	45-60	*7.40 ± 1.52*	*7.33 ± 0.46*	*10.09 ± 0.28*	*9.91 ± 1.14*			
plasma urea ($mmol \cdot l^{-1}$)	30	8.8 ± 0.5		6.6 ± 0.5		B_C	$D/D_L/D_O$	
	45	7.2 ± 0.6	3.9 ± 0.2	9.5 ± 0.6	5.1 ± 0.3			
	60	8.2 ± 1.1	3.5 ± 0.3	8.5 ± 0.2	4.3 ± 0.2			
plasma 2-amino N ($mmol \cdot l^{-1}$)	30	6.6 ± 0.3		6.1 ± 0.4		B_C	D_L	T_C
	45	6.4 ± 0.3	7.0 ± 0.2	7.5 ± 0.6	6.4 ± 0.1			
	60	6.1 ± 0.6	6.4 ± 0.2	5.7 ± 0.2	6.1 ± 0.2			

The data shown are the mean ± SEM of pooled 15-day (30-45 or 45-60 days) or means of individual results. All groups had 5-6 different animals.
*Statistical comparisons between the groups (three-way ANOVA): the symbols indicate that there is a significant ($P<0.05$) or highly significant —in **bold**— ($P<0.001$) effect of the given parameter: B = breed, D = diet and T = time; subindexed letters limit the significance to the indicated groups: $_R$ = reference diet, $_C$ = cafeteria diet; $_L$ = lean rats, $_O$ = obese rats; non-subindexed letters refer to the global significance of the effects of breed, diet or time. Only the significant relations are shown. The effects of breed or diet upon individual 15-day time stretches or between different age-groups are not shown.*

Alanine losses through urine were more marked in the second period of time for all groups, increased in cafeteria-fed rats and were also higher for obese rats, thus there is a 24-fold difference of concentration between the reference-fed 30 to 45–day lean rats and the cafeteria-fed 45 to 60–day obese rats, which secreted urine with almost 1 mM alanine. In contrast, the high levels of glycine observed in all groups showed very little change.

Hydroxyproline losses were low in all groups, being lower than the limit of detection for all obese rats fed the reference diet. Threonine concentrations increased in lean rats with cafeteria-feeding but decreased with time, whilst practically a reversed pattern was found in obese rats. For all groups, the concentrations of tyrosine and isoleucine were below the limit of detection of the method used, and other amino acids: phenylalanine, valine and leucine were very close to these limits.

Both lean and obese rats had higher urine urea levels when fed the reference than the cafeteria diet. In addition, obese rats showed higher urea levels than their lean counterparts. No differences were found between the first and second period urea concentrations in lean rats, but the obese showed higher levels in the second when fed the reference diet.

In Table 2 the net energy and nitrogen intake and the amount of nitrogen excreted in the urine are presented, together with the mean volumes of urine excreted and the plasma concentrations of urea and 2-amino nitrogen. There was a close parallelism between energy and nitrogen intake and the nitrogen excreted through the urine, with cafeteria-fed rats ingesting and excreting more than the

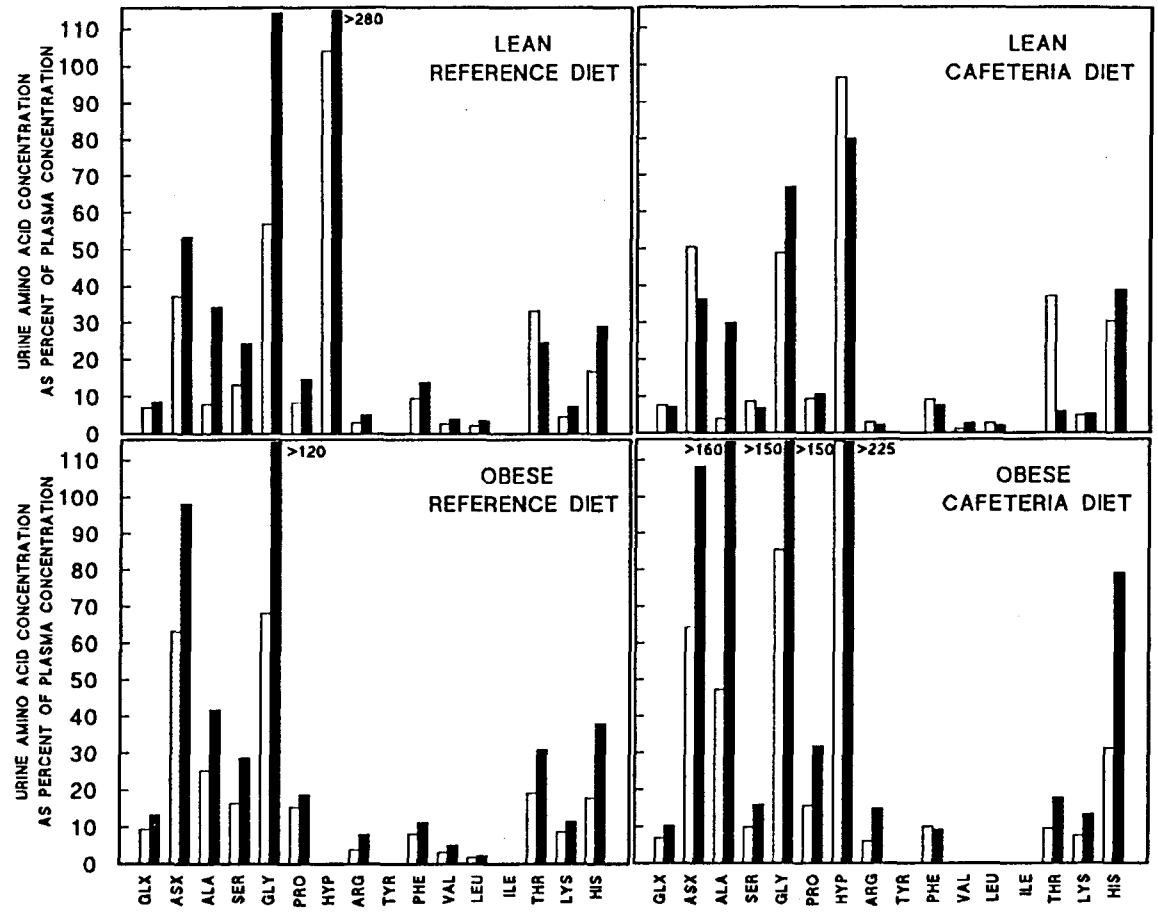


Figure 1

URINE AMINO ACID CONCENTRATIONS EXPRESSED AS PERCENT OF MEAN 15-DAY PLASMA AMINO ACID CONCENTRATIONS.

White columns: data for 30-45 day rats; black columns: data for 45-60 day rats. The values were calculated from data in table 2 and the means of plasma amino acids on days 30 and 45 for the first group and 45 and 60 for the second.

reference diet–fed, in addition, obese rats ate more and eliminated more nitrogen in the urine. The volume of urine excreted was higher for the second period studied than for the first, but no effects of obesity or diet were observed.

Plasma urea was lower in cafeteria-fed rats, obese rats fed the cafeteria diet had urea levels slightly higher than those of their lean counterparts. The changes observed in total plasma 2-amino nitrogen were less marked, with only small effects for diet, breed and time.

The ratios between mean plasma amino acids and urine amino acids are presented in Figure 1. In all groups, the losses of essential amino acids (plus arginine and tyrosine) were low compared with those of some non-essential. The exceptions were threonine and histidine, which were present in urine at concentrations ranging from 10 to 40 % of those found in plasma. Obese rats fed the cafeteria diet showed high histidine ratios, which were highest in the second 15-day period. The only situations where amino acids were more concentrated in urine than in plasma were found only in the second 15-day period, except for cafeteria-fed obese rats hydroxyproline. Alanine concentration was higher than 160 % that of plasma in cafeteria-fed obese rats. The levels of glycine were higher than 100 % for all groups except cafeteria-fed lean rats (80-100 %). Hydroxyproline reached high ratios in lean rats as well as in cafeteria-fed obese animals.

Table 3

Urinary amino acid losses of Zucker lean and obese rats fed reference and cafeteria diets

amino acid	age group	lean reference	lean cafeteria	obese reference	obese cafeteria	statistical comparisons breed	diet	time
Glx	30-45	0.15 ± 0.02	0.20 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	B/B_R		
	45-60	*0.19 ± 0.03*	*0.17 ± 0.02*	*0.13 ± 0.01*	*0.18 ± 0.02*			
Asx	30-45	0.33 ± 0.03	0.41 ± 0.03	0.35 ± 0.02	0.35 ± 0.05	B_C		T_O
	45-60	*0.36 ± 0.03*	*0.21 ± 0.02*	*0.38 ± 0.03*	*0.48 ± 0.04*			
Ala	30-45	0.65 ± 0.05	0.37 ± 0.02	1.45 ± 0.12	1.99 ± 0.52	B/B_C	D/D_O	$T/T_O/T_C$
	45-60	*1.67 ± 0.43*	*2.35 ± 0.29*	*2.03 ± 0.85*	*7.67 ± 2.65*			
Ser	30-45	0.43 ± 0.04	0.37 ± 0.02	0.35 ± 0.01	0.24 ± 0.02	B/B_R	D/D_L	$T/T_O/T_R$
	45-60	*0.64 ± 0.11*	*0.32 ± 0.03*	*0.44 ± 0.03*	*0.39 ± 0.04*			
Gly	30-45	1.74 ± 0.27	1.67 ± 0.09	0.99 ± 0.09	1.25 ± 0.14	B/B_R		
	45-60	*2.08 ± 0.53*	*1.89 ± 0.10*	*0.88 ± 0.15*	*1.41 ± 0.15*			
Pro	30-45	0.17 ± 0.02	0.19 ± 0.02	0.17 ± 0.02	0.23 ± 0.04	$B/B_R/B_C$	D/D_L	$T/T_O/T_C$
	45-60	*0.25 ± 0.03*	*0.19 ± 0.03*	*0.14 ± 0.01*	*0.45 ± 0.03*			
Hyp	30-45	6.72 ± 0.85	2.61 ± 0.43	0 ± 0	2.13 ± 0.57	B/B_R	$D/D_L/D_O$	
	45-60	*7.29 ± 0.99*	*2.24 ± 0.46*	*0 ± 0*	*3.45 ± 0.02*			
Arg	30-45	0.11 ± 0.00	0.10 ± 0.01	0.10 ± 0.02	0.14 ± 0.02	B/B_C	D_L/D_O	T_O
	45-60	*0.50 ± 0.02*	*0.07 ± 0.02*	*0.10 ± 0.01*	*0.21 ± 0.03*			
Tyr	30-45	0 ± 0	0 ± 0	0 ± 0	0 ± 0	—		
	45-60	*0 ± 0*	*0 ± 0*	*0 ± 0*	*0 ± 0*			
Phe	30-45	0.14 ± 0.03	0.13 ± 0.02	0.09 ± 0.01	0.11 ± 0.02			
	45-60	*0.16 ± 0.02*	*0.10 ± 0.01*	*0.13 ± 0.03*	*0.12 ± 0.02*			
Val	30-45	0.07 ± 0.01	0.28 ± 0.01	0.10 ± 0.02	0 ± 0	B_C	$D/D_L/D_O$	
	45-60	*0.09 ± 0.01*	*0.05 ± 0.01*	*0.11 ± 0.02*	*0 ± 0*			
Leu	30-45	0.03 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0 ± 0	B/B_C	D/D_O	T_R
	45-60	*0.04 ± 0.01*	*0.03 ± 0.00*	*0.04 ± 0.01*	*0 ± 0*			
Ile	30-45	0 ± 0	0 ± 0	0 ± 0	0 ± 0	—		
	45-60	*0 ± 0*	*0 ± 0*	*0 ± 0*	*0 ± 0*			
Thr	30-45	1.57 ± 0.49	3.27 ± 0.36	0.72 ± 0.04	0.40 ± 0.03	$B/B_R/B_C$	D_L	$T/T_L/T_C$
	45-60	*1.12 ± 0.16*	*0.42 ± 0.03*	*0.98 ± 0.11*	*0.80 ± 0.10*			
Lys	30-45	0.45 ± 0.04	0.40 ± 0.01	0.67 ± 0.04	0.40 ± 0.05	$B/B_R/B_C$	$D/D_L/D_O$	$T/T_O/T_R/T_C$
	45-60	*0.63 ± 0.05*	*0.34 ± 0.04*	*0.76 ± 0.05*	*0.61 ± 0.06*			
His	30-45	0.32 ± 0.05	0.63 ± 0.06	0.22 ± 0.01	0.44 ± 0.06		$D/D_L/D_O$	$T/T_O/T_C$
	45-60	*0.39 ± 0.03*	*0.64 ± 0.08*	*0.30 ± 0.03*	*0.76 ± 0.08*			

The data are expressed as percentage of absorbed (ingested minus lost in the faeces) amino acids. *Statistical comparisons between the groups (three-way ANOVA): the symbols indicate that there is a significant ($P<0.05$) or highly significant —in **bold**— ($P<0.001$) effect of the given parameter: B = breed, D = diet and T = time; subindexed letters limit the significance to the indicated groups: $_R$ = reference diet, $_C$ = cafeteria diet; $_L$ = lean rats, $_O$ = obese rats; non-subindexed letters refer to the global significance of the effects of breed, diet or time. Only the significant relations are shown. The effects of breed or diet upon individual 15-day time stretches are not shown.*

Table 3 shows the proportion of assimilated amino acids that are lost in the urine during the two studied periods. The results show that the overall loss of amino acids is very low, being zero or practically zero for most essential amino acids, and lower than 1 % for practically all except alanine, glycine and hydroxyproline.

Obesity resulted in lower urinary losses of glutamate plus glutamine, serine, glycine hydroxyproline and threonine, but increases of alanine and lysine. Cafeteria feeding increased alanine, proline and histidine excretion, diminishing that of serine. The differences between both 15-day periods were low, with an overall trend to higher losses in the 45-60 day period.

Discussion

The process of formation of urine is designed to prevent any significant losses of usable materials, however, the effectiveness of the reabsorption mechanisms is not complete for some essential strategic supplies, such as minerals. As for most metabolic substrates, their presence in urine in significant amounts is often an indication of altered homeostasis. Amino acids are found in urine either as free amino acids (5) or bond as peptides and —often— proteins (1). In humans, most of the unused hydroxyproline is lost in the urine in the form of oligopeptides (18). The presence of some amino acids or nitrogen-containing catabolites (e.g. indoleacetic acid) are often correlated with some physiological states such as pregnancy (3). The altered pattern of urinary amino acid excretion is thought to be a correlate from that of plasma —from which urine evolves— further modified by modulation of the renal amino acid transport systems (19).

The data presented confirm the effect of diet on the pattern and magnitude of urinary amino acids. The highest losses are found in animals receiving an energy-rich cafeteria diet, contrasting with the paucity of their urea (and total urinary nitrogen) loss). The cafeteria diet is known to diminish urinary nitrogen excretion, with lower urea production (20) despite higher circulating amino acid availability (10). This nitrogen conservation mechanisms are elicited by low dietary protein concentration (11), despite being compensated by hyperphagia up to fully adequate protein supply (14). The higher setting of nitrogen conservation processes observed in cafeteria feeding (11,20) are not correlated with diminished amino acid losses in urine, since they slightly increase. In obese rats, this higher loss is not primarily due to the raised plasma amino acids induced by cafeteria-feeding (10) since the urine versus plasma concentration ratios also increase.

The Zucker rat genetic obesity is characterized by hyperphagia and a consequent huge supply of nutrients, including protein (22), the resulting surplus nitrogen is eliminated in a large proportion as urine urea (11) and probably as elemental nitrogen as well (23). The pattern of urine amino acids, however, is not essentially different from that of lean rats, except for the practical absence of hydroxyproline. Since in many animals a large proportion of unused hydroxyproline is excreted in the form of peptides (18), and these have not been measured, the possibility remains that the difference between both breeds on that matter would rest in the form of elimination of this imino acid rather than in its actual utilization. Cafeteria-feeding resulted in the appearance of large amounts of free hydroxyproline in the urine of obese rats. It is, then, the combined effect of obesity and diet what alters the pattern of excretion of imino acids.

Obese rats fed the cafeteria diet excrete a large proportion of histidine, a condition similar to that encountered during pregnancy (7), when urinary excretion of amino acids is also altered (4). This allows to draw some parallelism between both situations, since in them there is an active lipogenesis from carbohydrate (24,25) and relative nitrogen preservation (10,11,26).

The relative uniformity of the volume of urine formed every day contrasts with the large differences in urea concentration, a major osmotic component, and the smaller changes in amino acids. Since we know the plasma and urine urea concentrations we can determine approximately the degree of concentration needed for converting the plasma ultrafiltrates into urine. Lean rats concentrated plasma about 120 to 130-fold when fed the reference and 180-190 the cafeteria diet. The corresponding figures for obese rats were: 160-170 for reference and 150-180 for cafeteria diet. This means that the extent of urine concentration is fairly homogeneous for all groups, the slight differences observed in amino acid levels between groups can be a consequence of the varying amplitude of the

concentration process. It must be borne in mind that even if the urinary concentration of alanine in cafeteria-fed obese rats is close to 1 mM, this represents that close to 80 times this same amount has had to be removed from the plasma ultrafiltrates to obtain the final concentration found in urine. The comparative figure for glutamate plus glutamine in the same experimental group would represent that in urine remained only close to 1/1,800th of these amino acids. For isoleucine, the extraction is even more complete, in the range of 1/20,000.

Despite some minor changes elicited by diet or breed the rat is able to retain most of the plasma amino acid —especially the essential ones— during the process of urine formation. The different patterns observed are only indicative of changes in the renal reabsorption process, suggesting that obesity and diet also affect renal functionality on that aspect.

Acknowledgements

Work supported by a grant PB88-0208 from the 'Dirección General de Investigación Científica y Técnica' from the Government of Spain. The authors acknowledge the technical assistance of Maria Reixach, Dr. Isidre Canals and Pilar Fernández from the Scientific and Technical Services of the University of Barcelona for their help in obtaining the aminograms. Thanks are given to Robin Rycroft for his help in the edition of the manuscript.

References

1. Bradley, M. and Schumann, G.B. (1986) In: *Clinical Diagnosis and Management by Laboratory Methods* (J.B. Henry, ed.), Saunders, Philadelphia.
2. Ulrich, K.L (1979) *Annu. Rev. Physiol.* 41: 181-187.
3. Page, E.W., Glendening, M.B., Diguam, W. and Harper, H.A. (1954) *Am. J. Obst. Gynecol.* 68: 110-118.
4. Miller, S., Ruttinger, V. and Macy, I.G. (1954) *J. Biol. Chem.* 209: 795-801.
5. Christensen, P.J., Oate, J.W., Schoenheyder, F. and Volquart, Z. (1957) *Scand. J. Clin. Lab. Invest.* 9: 54-61.
6. Passmore, R. and Eastwood, M.A. (1986) *Human Nutrition and Dietetics*, Churchill-Livingstone, Cambridge.
7. Pastor-Anglada, M. and Remesar, X. (1986) *Nutr. Res.* 6: 709-718.
8. Webber, W.A. (1967) *Can. J. Physiol. Pharmacol.* 45: 867-872.
9. Wallis, J.D. (1979) In: *Review of Physiological Chemistry* (H.A. Harper, V.W. Rodwell and P.A. Mayes, eds.), Lange, Los Altos.
10. Rafecas, I., Esteve, M., Remesar, X. and Alemany, M. (1991) *Biochem. Internat.* 25: 797-806.
11. Esteve, M., Rafecas, I., Remesar, X. and Alemany, M. (1992) *Int. J. Obesity* 16: 237-244.
12. Harris, R.B.S., Tobin, G. and Henry, G.R. (1988) *J. Nutr.* 118: 503-514.
13. Ruiz, B., Felipe, A., Casado, J. and Pastor-Anglada, M. (1991) *Biochem. J.* 180: 367-372.
14. Prats, E., Monfar, M., Castellà, J., Iglesias, R. and Alemany, M. (1989) *Physiol. Behav.* 45: 263-372.
15. Fawcett, J.K. and Scott, J.L. (1960) *J. Clin. Pathol.* 12: 156-163.
16. Samejima, K., Dairman, W., Stone, J. and Udenfriend, S. (1971) *Anal. Biochem.* 42: 237-247.
17. Heinrikson, R.L. and Meredith, S.C. (1984) *Anal. Biochem.* 136: 65-74.
18. Altman, P.L. and Dittner, D.S. (1974) *Biology Data Book* vol.3, 2nd.ed., Federation of American Societies for Experimental Biology, Washington.
19. Bergeron, M. and Morel, F. (1969) *Am. J. Physiol.* 216: 1139-1144.
20. Barber, T., Viña, J.R., Viña, J. and Cabo, J. (1985) *Biochem. J.* 230: 675-681.
21. Esteve, M., Rafecas, I., Remesar, X. and Alemany, M. (1992) *Biochem. Int.* 26: 687-694.
22. Radcliffe, J.D. and Webster, A.J.F. (1978) *Br. J. Nutr.* 309: 483-492.
23. Costa, G., Ullrich, L., Kantor, F. and Holland, J.F. (1968) *Nature* 218: 546-551.
24. Humprey, J.L., Talbot-Childs, M., Montes, A. and Knopp, R.H. (1980) *Am. J. Physiol.* 239: E81-E82.
25. Goldbole, V., York, D.A. and Bloxham, D.B. (1978) *Diabetologia* 15: 41-44.
26. Beaton, G.H. (1957) *Arch. Biochem. Biophys.* 67: 1-9.

6 DISCUSSIÓ

Acreció de reserves energètiques

En l'estudi de la deposició dels diferents components energètics es pot observar que, en tots els casos la dieta de *cafeteria* comporta un increment de l'acumulació de lípids i també, encara que menys important, de proteïnes. Aquesta alta eficiència en la deposició de greix es predominant en la rata obesa fa/fa, i màxima quan està sotmesa a dieta de *cafeteria*, assolint, en un mes de tractament, un increment de pes de quatre vegades l'inicial. Les diferències entre els lípids ingerits i els dipositats en els grups control, suggereixen una elevada taxa lipogènica a partir del glúcids i/o proteïnes. Aquest tret comença a fer-se important en el segon període estudiat en ambdós fenotips prims, mentre que en les rates obeses es present ja des d'un primer moment. En canvi, en el tractament amb dieta de *cafeteria* no s'observa aquesta elevada taxa lipogènica, possiblement degut a que la dieta aporta una major quantitat de greix que es diposita directament. Les rates obeses alimentades amb dieta de *cafeteria* mostren una eficiència de deposició de lípids més elevada que les rates primes, però menor que elles mateixes quan menjaven dieta control.

També s'observa una relativa uniformitat en l'eficiència de deposició de proteïnes entre els diferents grups, però cal destacar que aquesta es més alta en tots aquells grups de rates tractades amb dieta de *cafeteria*. Aquesta uniformitat, incloent-hi la rata fa/fa, ens indica que contràriament al que s'ha descrit (Dunn i Hartsook, 1980), la rata Zucker fa/fa no té una menor deposició de proteïnes, sinó que la seva massa prima en termes absoluts és equivalent a la de la control, però que relativament és més petita degut al més gran contingut en greix.

La relació entre reserves de greix/proteïnes és més elevada en la rata obesa, i la dieta de *cafeteria* té un efecte més petit que en els altres grups en fer incrementar-la, possiblement perquè estigui ja prop del límit. Aquests índex ens indica que el comportament quant a la deposició de lípids, és diferent en les rates fa/fa que el trobat en les hiperfàgiques per dieta de *cafeteria*, doncs aquestes últimes acumulen relativament més proteïnes.

Metabolisme nitrogenat

En l'estudi del balanç de nitrogen, s'observa que en l'obesitat induïda mitjançant dieta de *cafeteria*, en la soca Wistar, la ingesta de nitrogen és més baixa en proporció al total de l'energia ingerida, que en el grup control, però en canvi la retenció és similar. Això, sembla donar-se gràcies a una absorció de N a nivell intestinal més eficient, doncs es recupera menys N del ingerit en la femta, quan es compara amb les rates control. També hi ha una menor utilització del N absorbit, amb una activitat del cicle de la urea disminuïda (Barber et al, 1985), excretant-se menys urea en valors absoluts, mentre que la proporció de urea en l'orina no varia en ambdues dietes. Conseqüentment hi ha una major retenció de N en el cos de l'animal. Una possible explicació d'aquest fet podria fer pensar que la proteïna de la dieta de *cafeteria* és d'una millor qualitat, de manera que aportaria més quantitat d'aminoàcids essencials per quantitat de proteïna, essent necessàries menys quantitat per aconseguir els nivells de requeriment. De manera , que no caldria oxidar l'excés d'aminoàcids no necessaris aportats per proteïnes de més baixa qualitat.

El fenotip prim de la soca Zucker mostra el mateix comportament que la rata Wistar al sotmetre's a dieta de *cafeteria*. Contràriament, en el fenotip obès (fa/fa)es presenta un comportament diferent, amb una menor eficiència de retenció de N, trobant-se una

proporció de N de la dieta en femta lleugerament més elevada que en la rata control prima. Així doncs, contràriament al que passa en l'alimentació amb dieta de *cafeteria*, l'obesitat genètica present en la rata fa/fa té una menor eficiència d'absorció intestinal del N. La diferència més marcada entre ambdós models i que afecta a la menor retenció, són els elevats nivells d'excreció d'urea en orina en la rata obesa fa/fa, doncs, a més de que la quantitat d'urea excretada és més alta, també ho és la proporció d'urea en l'orina, trobant-se valors pràcticament del 100%. Aquests factors colaboren a que hi hagi una menor retenció del N ingerit en el cos de l'animal obès, tot i que en termes absoluts la quantitat de N retingut és equivalent a la dels animals prims. Quan la rata obesa es sotmet a dieta de *cafeteria*, si bé mostra uns efectes aditius en quan el metabolisme energètic en general, es a dir augmenta més de pes i guanya lípids, pel que fa al metabolisme nitrogenat no es comporta de la mateixa manera. En aquest cas el efectes d'ambdues obesitats són més aviat compensatoris, doncs la rata fa/fa alimentada amb dieta de *cafeteria* incrementa la seva absorció intestinal de N, baixa el catabolisme del N i augmenta la proporció del N ingerit que es retingut en el cos de l'animal, tot i una ingesta proteica més baixa que quan menja dieta control.

Si suposem que la rata obesa manté la seva temperatura corporal a expenses del seu metabolisme basal, tal com s'ha suggerit (Himms-Hagen, 1989), donat que té un sistema termogènic defectuós en tenir un teixit adipós marró no totalment funcional, aleshores, es veu obligada a incrementar la seva ingesta per tal d'incrementar la seva taxa metabòlica basal. Si això és així, un increment d'ingesta amb la dieta de referència comporta alhora una elevada ingesta de proteïna no necessària, de la que s'oxida la major part, mentre que amb la dieta de *cafeteria* pot escollir i tendeix a menjar més greix, que té un contingut energètic més elevat, i a reduir la ingesta de proteïna a uns nivells més pròxims als requeriments.

En aquests estudis trobem que una part del nitrogen ingerit no el recuperem ni en les femtes, ni en orina, ni tampoc es retingut. En principi cal revisar la metodologia emprada, així una vegada convençuts que els errors metodològics (sobre-estimació de la ingesta, pèrdues de pels, NH^+_4 pels pulmons) no poden explicar-nos el total del N no trobat, que és d'un 10 % a un 27 % en controls i *cafeteria* respectivament, cal plantejar-se que potser hi hagi algun altre via d'utilització de N no coneguda. S'ha plantejat ja en diverses ocasions la possibilitat de producció de nitrogen gas, en aquest sentit hi ha treballs (Costa et al, 1968, Cissik, 1972) on es descriu que en ratolí i home es produeix un alliberament de N_2 i que aquest pot representar fins un 10% del N de la ingesta, i a més, sembla que la producció estigui correlacionada amb la quantitat de proteïna present en la dieta. Nosaltres trobem que en les rates que mengen dieta control hi ha a prop del 10% de N no recuperat, mentre que les que menjaven dieta de *cafeteria* podia representar fins el 20-27%, això ens va fer suposar que potser més que la quantitat seria la qualitat de la proteïna,el factor implicat en l'increment de la formació de N_2.

Quant l'estudi del balanç d'aminoàcids, s'han ratificat els trets vistos en el balanç global de N, de manera que les rates sotmeses a dieta de *cafeteria* tenen una més gran eficiència d'absorció, eliminant-se menys aminoàcids per la femta, amb una retenció més elevada i trobant-se en orina unes pèrdues d'aminoàcids mínimes i lleugerament més baixes que en utilitzar-la dieta control, en la rata Wistar. Això, és un fet que s'observa en general per tots els aminoàcids, tant essencials com no essencials, tot i que les taxes d'absorció dels diferents aminoàcids no és uniforme, el que pot estar condicionat per canvis en els sistemes de transport intestinals induïts per la dieta.

Un tret important que s'ha pogut observar gràcies al balanç detallat de cada aminoàcid, és que curiosament les rates alimentades amb dieta de *cafeteria* seleccionen una dieta que finalment mostra el mateix patró d'aminoàcids de la dieta control. La dieta control ha estat molt ben estudiada per tal de que aporti la proporció adequada pel creixement i manteniment de la rata (Rogers, 1979), aleshores veiem que aquest animal es capaç de controlar d'una manera molt precisa la seva ingesta proteica. Això s'observa amb els tres tipus de rates estudiades, incloent-hi la rata Zucker obesa indicant que aquesta no té una alteració en el control de la ingesta nitrogenada i que sembla doncs que aquest control s'exerceix independentment del control energètic global. D'altra banda, aquests resultats ens indiquen que aquest model dietari no és deficient amb un aport proteic adequat com s'ha suggerit (Moore, 1987), i que tampoc és certa la suposició que havíem fet prèviament de que la dieta de *cafeteria* aportava unes proteïnes de més alta qualitat, ja que la proteïna que s'ha ingerit és exactament igual en la proporció d'aminoàcids que la ingerida en la dieta control. Això implica doncs, que la més gran absorció i més gran retenció en la dieta de *cafeteria* no es pot explicar per la qualitat de la proteïna ingerida. D'altres causes que puguin afectar aquest millor aprofitament de nitrogen en la dieta *cafeteria* podrien ser per exemple la proporció de fibra o bé la quantitat de proteïna respecte l'energia global ingerida. En aquest sentit troben que la dieta control conté un 4,3 % de fibra mentre que la de *cafeteria* un 3 %, no hi ha doncs una diferència molt marcada tot i que segur que té alguna influència en la velocitat del trànsit intestinal, no creiem que sigui aquest factor el condicionant d'un més gran aprofitament del N. Si analitzem la diferent proporció de proteïnes, glúcids i greixos en les dues dietes trobem que les proteïnes són un 21,7 % (1,55 ± 0,02 MJ) i 14.3 % (1,27 ± 0,02 MJ), els glúcids 70.3 % (5,10 ± 0,07 MJ) i 59.3 % (5,26 ± 0,02 MJ) i els lípids 7,9 % (0,56 ± 0,01 MJ) i 27,5 % (2,44 ± 0,11 MJ) de la dieta control i de la de *cafeteria* seleccionada per la rata Wistar respectivament, dades que són pràcticament iguals en el cas de les rates de la soca Zucker. D'aquestes dades veiem que la diferencia entre ambdues dietes, pel que fa al contingut de proteïnes i glúcids no és excessivament diferent, mentre que hi ha una diferència molt marcada quant la quantitat de greix ingerit, per això suggerim que és aquest el factor que determina la millor utilització de les proteïnes en la dieta de *cafeteria*.

Quan mirem la taxa de acreció proteica s'intueix que està fortament afectada per la dieta, però essent-ho molt més per l'edat, o potser pel tamany de l'animal, ja que veiem que les rates que mengen dieta de *cafeteria* creixen més ràpid de manera que en el segon període estudiat baixa considerablement la seva taxa de deposició proteica, mentre que la rata amb dieta control creix més lentament i manté més uniforme la seva taxa de deposició proteica durant tot el període estudiat. En aquests sentit explicaríem que en la rata Wistar amb dieta de control presenti una composició corporal més rica amb Ala, Gly i Pro que la rata amb dieta de *cafeteria* en l'últim període, ja que al ser més prima perquè creix més lentament, presenta una proporció de pell més gran en relació al volum, i al pell és rica amb col·làgena, proteïna que conté grans proporcions d'aquests aminoàcids.

Ambdues obesitats, genètica i induïda per la dieta de *cafeteria* es caracteritzen per mostrar un elevat nivell de ingesta, de pes i un increment en l'acumulació de greix, a més, l'obesitat en la rata Zucker mostra una producció de calor més baixa que les primes, mentre que les rates de *cafeteria* tenen unes altes taxes de producció de calor en comparació a les alimentades amb dieta estàndard. Així,l'obesitat genètica és energèticament més eficient que l'obesitat nutricional. Pel que fa al metabolisme proteic la rata geneticament obesa perd quantitats importants de nitrogen en femtes i manté una elevada activitat del cicle de la urea excretant més aminoàcids per orina que les controls, en canvi ja hem comentat que les rates alimentades amb dieta de *cafeteria* es comporten

en sentit contrari. Pel que fa a la taxa d'acreció de proteïna en la rata Zucker obesa es manté elevada durant tot el període estudiat, però, quan mengen dieta de *cafeteria* incrementen la taxa d'acreció en el primer període però la disminueixen fins el nivell de les seves controls en segon període estudiat. En tots els casos tractats amb dieta de *cafeteria* sembla ser que el nitrogen no trobat incrementa en disminuir la taxa de creixement, es a dir en el segon període, efecte combinat amb una major disponibilitat d'aminoàcids.

Hi ha encara altres diferències en el metabolisme dels aminoàcids en ambdós tipus d'obesitat. Així, en la rata Wistar i Zucker primes, sotmeses a dieta de *cafeteria* el nivell d'aminoàcids en plasma es més elevat que en els controls, fet que es conseqüent amb el mecanisme general d'estalvi de nitrogen manifest en aquest estat, i que és similar al que es dona en situacions de dietes deficients en proteïna, dietes diluïdes i el dejuni. La dieta de *cafeteria* pot causar hiperinsulinèmia i resistència a la insulina disminuint l'oxidació dels aminoàcids de cadena ramificada. La obesitat de la rata Zucker mostra uns nivells d'aminoàcids en plasma més elevats en general que les controls primes, essent els de cadena ramificada els que mostren diferències més significatives, fet que pot estar relacionat amb la hiperinsulinèmia i resistència a la insulina d'aquestes rates. Quan es sotmet a dieta de *cafeteria* contràriament a l'esperat, el nivell d'aminoàcids en plasma disminueix en general, excepte per la Thr, Ser, Pro i Hyp que mostren un petit augment.

En la rata Zucker fa/fa tant amb dieta control com amb dieta de *cafeteria* s'observa que amb l'edat incrementen fins al doble els nivells de Phe, mentre que els de Tyr baixen a la meitat, fet que podria indicar un impediment d'aquest animal per convertir la Phe en Tyr, ja que el contingut d'aquests aminoàcids en la dieta són similars als de la rata prima.

Pel que fa referència a l'excreció d'aminoàcids per orina sembla que la soca Zucker alimentada amb dieta de *cafeteria* es comporta de manera diferent a la soca Wistar, ja que tant en primes com en obeses els nivells d'aminoàcids trobats en orina són més alts que en dieta control, tot i que la producció d'urea disminueix. En la rata obesa aquestes pèrdues no poden ser conseqüència d'una pujada en els nivells plasmàtics, a més la relació aminoàcids d'orina respecta plasma també incrementa. El patró d'aminoàcids en orina no són diferents entre la rata prima i l'obesa, excepte per la Hyp que no es troba en l'orina de la rata obesa amb dieta control. La Hyp no utilitzada pot ser excretada en forma d'aminoàcid i de pèptid, podria ser que aquesta última forma fos la utilitzada per la rata obesa. La Hyp es present en orina de la rat fa/fa amb dieta de *cafeteria* de manera que podria ser que l'efecte combinat de l'obesitat i la dieta afecti el patró d'excreció dels iminoàcids.

De l'efecte de la dieta de *cafeteria* en resulta un increment significatiu en l'excreció per orina de His de manera similar al trobat en la gestació (Pastor-Anglada i Remesar, 1986b). Es pot establir un paralelisme entre ambdues situacions, presentant una elevada taxa lipogènica a partir dels glúcids i un estalvi de nitrogen que coincideix amb un transport d'aminoàcids alterat.

7 CONCLUSIONS

1. El subministrament d'una dieta hipercalòrica (hiperlipídica), de tipus *cafeteria*, indueix un augment en l'eficiència de deposició de proteïnes, tant en animals prims com genèticament obesos.

2. El destí del nitrogen incorporat a l'organisme, implica l'existència d'una fracció (no atribuible) que no es detectada per la metodologia convencional i que pot representar més d'un 10% del N ingerit. Aquesta fracció es modulable per la dieta.

3. L'obesitat genètica condiciona una diferent disponibilitat del nitrogen de la dieta, en donar-se una menor absorció intestinal i un augment del nitrogen ureic en l'orina, tot comparant-lo amb els animals prims. Aquesta situació es parcialment normalitzada en subministrar a aquests animals una dieta tipus *cafeteria*.

4. El consum d'una dieta de tipus *cafeteria* presuposa la ingestió de la mateixa quantitat i qualitat d'aminoàcids que es dona en consumir una dieta estàndard. Aquest fet no ve influenciat per les característiques genètiques dels animals d'experimentació.

5. Els animals alimentats amb una dieta de tipus *cafeteria* tenen augmentada la capacitat d'absorció intestinal d'aminoàcids, sense que es pugui establir un comportament diferencial per aminoàcids essencial i no essencials, malgrat la manca d'uniformitat detectada.

6. Els animals genèticament obesos es caracteritzen per absorbir menys aminoàcids que els seus controls prims. El tractament amb una dieta de tipus *cafeteria* apropa aquest comportament al de les rates primes, excepte pel cas d'alguns aminoàcids essencials.

7. L'administració d'una dieta de cafeteria als animals de la soca Zucker, treu a la llum l'anormal capacitat de filtració renal d'aquest animals, en excretar més quantitat d'aminoàcids que en ser alimentats amb dieta estàndard.

8. L'obesitat genètica comporta una metabolització diferencial dels aminoàcids, doncs malgrat presentar uns nivells plasmàtics similars als dels animals prims, no augmenten en donar-lis dieta de cafeteria, fet que es dóna en els animals prims.

8 BIBLIOGRAFIA

Ahmad, I., Steggles, A.W., Carrillo, A.J., Finkelstein, J.A. Developmental changes in levels of growth homone mRNA in Zucker rats. J. Cell. Biochem., 43: 59-66, 1990

Alemany, M. The etiologic Basis for the classification of obesity. Progress in food and Nutrition Science, 13: 45-66, 1989

Anand, B.K., Brobeck, J.R. Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med., 24: 123-146, 1951

Arase, K., Fisler, J., Shargill, N., York, D., Bray, G. Intracerebroventricular infusions of 3-hydroxybutirate and insulin in a rat model of dietary obesity. Am. J. Physiol., 255: R974-R981, 1988

Arase, K., Shargill, N.S., Bray, G.A. Effect of corticotropin releasing factor on genetically obese (fatty) rats. Physiol. Behav., 45: 1-6, 1989

Assimacopoulos-Jeannet, F. & Jeanrenaud, B. The hormonal and metabolic basis of experimental obesity. Clin. Endodrinol. Metab., 5: 337-365, 1976

Astrup, A. Human obesity and brown fat metabolism. 7th International Symposium Pharmacology of Thermoregulation. Thermogenesis: Research and clinical applications, Odense, Denmark 1988. Lomax, Schönbaum eds; pp. 26-30 Karger, Basel, 1989

Atwater, W.O., Rosa, E.B. Description of a new respiration calorimeter adn experiments on the conservation of energy in the human body. Bull. U.S. Dep. Agriculture, 63, 1899

Bach, A.C., Babayan, V.K. Medium chain triglycerides: an update. Am. J. Clin. Nutr., 36: 950-955, 1982.

Baile, C.A., McLaughlin, C.L., Della-Fera, M.A. Role of cholecystokinin and opioid peptides in control of food intake. Physiol. Rev., 66: 172-234, 1986

Bailey, C.J., Day,C., Bray, G.A., Lipson, L.G., Flatt, P.R. Role of adrenal glands in the development of abnormal glucose and insulin homeostasis in genetically obese (ob/ob) mice. Horm. Metab. Res., 18: 357-360, 1986

Barbato, A.L., Landau, R.L. Testosterone deficiency of morbid obesity. Clin. Res., 22: 647A-651A, 1974

Barber, T., Viña, J.R., Viña, J., Cabo, J. Decreased urea synthesis in the cafeteria-diet-induced obesity in the rat. Biochem. J., 230: 675-681, 1985

Bateson, W. The present state of knowledge of colors herdity in mice and rats. Proc. Zool. Soc. London, 2: 71-99, 1903

Batt, R.A.L., Harrison, G.A. Features of the "adipose" mouse. (abstr.) J. Hered., 15: 335, 1960

Batt, R.A.L., Harrison,G.A. The reproductive system of the adipose mouse. J. Hered., 54: 135-138, 1963

Batt, R.A.L. Abnormal dentition and decrease in body weight in the genetically obese mouse (genotype obob). Int.J. Obesity, 2: 457-462, 1978

Bazin, R., Planche, E., Dupuy, F., Krief, S., Lavau, M. Deprivation of corticosterone does not prevent onset of obesity in Zucker fa/fa pups. Am. J. Physiol., 252: E461-E466, 1987

Bazin, R., Etève, D., Lavau, M. Evidence for decreased GDP binding to brown-adipose-tissue mitochondria of obese Zucker (fa/fa) rats in the very first days of life. Biochem. J., 221: 241-245, 1984

Bazin, R., Krief, S., Dupuy, F., Lavau, M. Effect de la surrénalectomie sur la capacité de thermogénèse du tissu adipeux brun et de le développement de l'obésié chez le rat fa/fa. Reprod. Nutr. Dev., 26: 643-648, 1986

Bazin, R., Lavau, M., Guichard, C. Development of fatty-acid synthetic capacity in interscapular brown adipose tissue during suckling in genetically obese Zucker rats. Biochem. J., 216: 543-549, 1983

Bazin, R., Lavau, M. Development of hepatic and adipose tissue lipogenic enzymes and insulinaemia during suckling and weaning on to a high-fat diet in Zucker rats. J. Lipids Res., 23: 839-849, 1982

Bell, G.E., Stern, J.S. Evaluation of body composition of young obese and lean Zucker rats. Growth, 41: 63-80, 1977

Benzinger, T.H., Kitzinger, C. Direct calorimetry by means of the gradient principle. Review Scientific Instruments, 20: 849-860, 1949

Bernardis, L.L., Goldman, J.K. Origin of endocrine-metabolic changes in the weanling rat ventromedial syndrome. J. Neurosci. Res., 2: 91-116, 1976

Bernardis, L.L. Disruption of diurnal feeding and weight gain cycles in weanling rats by ventromedial and dorsomedial hypothalamic lesions. Physiol. Behav. 10: 855-861,1973

Bingham, S.A., Cummings, J.H. Urine nitrogen as an independent validatory measure of dietary intake: a study of nitrogen balance in individuals consuming their normal diet. Am. J. Clin. Nutr., 42: 1276-1289, 1985

Björntorp, P. Adipose tissue in obesity (Willendorf Lecture). In: Recent advances in obesity research, eds Hirsch, J., Van Itallie JB., London, 1985

Blaxter, K.L. Energy metabolism in animals and man. Cambridge University Press, Cambridge, 1989

Blaxter, K.L., Wainman, F.W. Enviromental temperature and the energy metabolism and heat emission of steers. J. Agricul. Sci., Cambridge, 56: 81-90, 1961

Bloom, W.L., Eidex, M.F. Inactivity as a major factor in adult obesity. Metab. Clin. Exp. Med., 16: 679-681, 1967

Blundell, J. Pharmacological approachesto appetite supression. Trends in Pharmacol.l Sci.,

4: 147-157, 1991

Boulangé, A., Planche, E., de Gasquet, P. Onset and development hypertriglyceridemia in the Zucker rat. Metabolism, 30: 1045-1052, 1982

Boulangé, A., Planche, E., deGasquet, P. Onset of genetic obesity in the absence of hyperphagia during the first week of life in the Zucker rat. J. Lipid Res., 20: 857-864, 1979

Bray, G.A. Effect of caloric restriction on energy expenditure in obese patients. Lancet, 2: 397-398, 1969

Bray, G.A., York, D.A. Hypothalamic and genetic obesity in experimental animals, an autosomic and endocrine hypothesis. Physiol. Rev., 59: 719-809, 1979

Bray, G.A. Weight homeostasis. Annu. Rev. Med., 42: 205-216, 1991

Bray, G.A., York, D.A., Swelrdloff, R.S. Genetic obesity in rats. I: The effects of food restriction on body composition and hypothalamic function. Metabolism, 22: 435-442, 1973

Bray, G.A., York, D.A., Yukimura, Y. Activity of $(Na^+ + K^+)$-ATPase in the liver of animals with experimental obesity. Life Sci., 22: 1637-1642, 1978

Bray, G.A. Obesity a disease of nutrient or energy balance?. Nutr. Rev., 45: 33-43, 1987

Bray, G.A. A molecular appoach to genetic and hypothalamic obesity. Obesity: Towards a Molecular Approach, G.A. Bray, D. Riequier and B.M. Spiegelmam eds., UCLA Symposia on molecular and cellular biology, 132: 1-15, Wiley-Liss, Inc., New York, 1990.

Bray, G.A. McCollum award lecture. Genetic and hypothalamic mechanisms for obesity-finding the needle in the haystack. Am. J. Clin. Nutr., 50: 891-902, 1989

Bray, G.A. Treatment for obesity: A nutrient balance/nutrient partition approach. Nutr. Rev., 49: No 2, 33-45, 1991

Bray, G.A., York D.A. Genetically transmited obesity in rodents. Physiol. Rev., 51: 598-646, 1971

Bray, G.A., Campfield, L.A. Metabolic factors in the control of enrgy stores. Metabolism, 24: 99-117, 1975

Bray, G.A. Regulation of energy balances: Studies on genetic, hypothalamic and dietary obesity. Proc. Nutr. Soc., 41: 95-108, 1982

Brobeck, J.R. Mechanism of development of obesity in animals with hypothalamic lesions. Physiol., Rev. 26: 541-559, 1946

Brobeck, J.R. In The body weigth regulatory system: Normal and disturbed mechanisms. Luigi, Cioffi eds. Raven Press New York, 1981

Brosnan, J.J., Man, K.-C., Hall, D.E., Colbourne, S.A., Brosman, M.E. Inter-organ metabolisme of amino acids in streptozotocin-diabetic ketoacidolic. Am. J. Physiol., 244: E151-E158, 1983

Bukowiecki, L.J., Folléa, N., Lupien, J., Paradis, A. Metabolic relationships between lipolysis and respiration in rat brown adipocites. The role of long chain fatty acids as regulators of mitochondrial respiration and feedback inhibitors of lipolysis. J. Biol. Chem., 256: 12840-12848, 1981

Butler, L., Gerritsen, G.C. A comparison of the modes of inheritance of diabetes in the Chinese hamster and the KK mouse. Diabetologia, 6: 163-167, 1970

Cabanac, M. Temperature regulation. A. Rev. Physiol., 37: 415-439, 1975

Calloway, D.H., Odell, A.F., Margen, S. Sweat and miscellaneous nitrogen losses in human balance studies. J. Nutr., 101: 775-786, 1971

Campbell, J.A. *Evaluation of protein quality*. NAS-NRC Publ. 31: 1100, 1963

Campfield, L.A., Brandon, P., Smith, F.J. On-line continuous measurement of blood glucose and mal pattern in free-feeding rats: the role of glucose in meal initiation. Brain Res. Bull., 14:605-616, 1985

Carpenter, K.J., Mayer, J. Physiologic observations of yellow obesityin the mouse. Am. J. Physiol., 193: 499-504, 1958

Casado, J., Remesar, X., Pastor-Anglada, M. Hepatic uptake of amino acids in the late-pregnant rats. Biochem. J., 248: 117-122, 1987

Casper, R.C. The patophysiology of anorexia nervosa and bulimia nervosa. Ann. Rev. Nutr., 6: 299-316, 1986

Castonguay, T.W., Dallman, M.F., Stern, J.S. Some metabolic and behavioral effect of adrenalectomy on obese Zucker rats.Am. J. Physiol., 251: R923-R933, 1986

Cattabeni, F., Maggi, A., Monduzzi, M., De Angelis, L., Racagni, G. GABA: Circadian fluctuation in rat hypothalamus. J. Neurochem., 31: 565-569, 1978

Cissik, J.H., Johnson, R.E., Rokosch, D.K. Production of gaseous nitrogen in human steady-state conditions. J. Appl. Physiol., 32: 155-159, 1972

Clausen, T., Hansen, O. *The Na^+-K^+ pump, energy metabolism, and obesity*. Biochem. Biophys. Res. Commun., 104: 357-362, 1982

Cleary, M.P., Vasselli, J.R., Grenwood, M.R.C. Development of obesity in Zucker obese (fa/fa) rat in absence of hyperphagia. Am. J. Physiol., 238: E284-E292, 1980

Closa, D., Gomez-Sierra, J.M., Latres, E., Alemany, M., Remesar, X. Short term oscillations of core temperature and thermogenia organs blood flow in the rat. Exp. Physiol., 1992 en premsa

Closa, D., Alemany, M., Remesar, X. In vivo measurement of Wistar and Zucker fa/fa rat organ temperatures, effect of cold exposure. J. Thermal. Biol., 17: 83-88, 1992

Cohn, C., Joseph, D. Influence of body weiht and body fat on appetite of normal lean and obese rats. Yale J. Biol. Med., 34: 598-601, 1962

Coleman, D.L., Hummel, K.P. Hyperinsulinemia in pre-weaning diabetes (db) mice. Diabetologia, 10: 607-610, 1974

Coleman, D.L. Obesity and diabetes. Two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia, 14: 141-148, 1978

Coscina, D.V., Nobrega, J.N. Anorecty potency of inhibiting GABA transaminase in brain: Studies of hypothalamic, dietary and genetic obesities. Int. J. Obes., 8: 191-200, 1984

Costa, G. Hypothetical pathway of nitrogen metabolism. Nature, 188: 549-552, 1960

Costa, G., Ullrich, L., Kantor, F., Holland, J.F. Production of elemental nitrogen by certain mammals including man. Nature, 218: 546-551, 1968

Cruce, J.A.F., Thoa, N.B., Jacobowitz, D.M. Catecholamines in the brains of genetically obese rats. Brain Res., 101: 165-170, 1976

Chan, C.P., Stern, J.S. Adipose tissue lipoprotein lipase in insulin-treated diabetic lean and obese Zucker rats. Am. J. Physiol., 242: E445-450, 1982

Cohn, C., Joseph, D. Changes in body composition with force feedig. Am. J. Physiol., 196: 965-968, 1959

Danforth, E., Horton, E.S., O'Connell, M. et al. Dietary-induced alterations in thyroid hormone metabolism during overnutrition. J. Clin. Invest., 64: 1336-1347, 1979

Davis, T.R.A., Mayer, J. Imperfect homeothermia in the hereditary obese-hyperglycemic syndrome of mice. Am. J. Physiol., 177: 222-226, 1954

de Boer, J.O., van Es, A.J.H., Roovers, L.C.A., van Raaj, J.M.A., Hautrast, J.G.A.J. Adoption of energy metabolism of overweight woman to low-energy intake, studied with whole-body calorimeters. Am. J. Clin. Nutr., 44: 585-595, 1986

Deb, S., Martin, R.J., Hershberger, T.V. Maintenance requirements and energetic efficiency of lean and obese Zucker rats. J. Nutr., 106: 191-197, 1976

Deutch; A.Y., Martin, R.J. Mesencephalic dopamine modulation of pituitary and central β-endorphin regulation to food intake regulation. Life Sci., 33: 281-287, 1983

Dietz, W.H., Gortmaker, S.L. Factors within the physical environment associet with childhood obesity. Am. J. Clin. Nutr., 39: 619-624, 1984

Donhoffer, S.F., Vonotzki, J. The effect of thyroxine on food intake and selection. Am. J. Physiol., 150: 334-339, 1947

Dourish, C., Rycroft, W., Inversen, S. Postponement of saciety by blockade of brain cholecystokinin (CCK-B) receptors. Science, 9: 1509-1511, 1989

Dugail, I., Quignard-Boulangé, A., Bazin, R., Le Liepvre, X., Lavau, M. Adipose-tissue-specific increase in glyceraldehyde-3-phosphate dehydrogenase activity and mRNA amounts in suckling pre-obese Zucker rats. Biochem. J., 254: 483-487, 1988

Dunn, M.A., Hartsook, E.W. Comparative amino acid and protein metabolism in obese and non-obese Zucker rats. J. Nutr., 110:1865-1879, 1980

Durbin-Naltchayan, S., Bouhnik, J., Michel, R. Tyroid status in the obese syndrome of rats. Horm. Metab. Res., 15: 547-549, 1983

Eaton, G.J., Green, M.M. Giant cell differentiation and lethality of homozygous yellow mouse embryos. Genetics, 34: 155-166, 1963

Elwyn, D., Launder, W.J., Parikk, H.C., Wise, E.M. Role of plasma and erythrocyites in interorgans transpot of amino acids in dogs. Am. J. Physiol., 222: 1333-1342, 1972

FAO/WHO/UNU. Energy and protein requirements. Tech. Rep. Ser. No 724, World Health Organization, Genova, 1985

Felig, P., Wahren, J. Amino acid metabolism in exercising man. J.Clin. Invest., 50: 2703-2714, 1971

Filkelstein, J.A., Jervois, P., Menadue, M., Willough, J.O. Growth hormone and prolactin secretion in geneticlly-obese Zucker rats. Endocrinology, 118: 1233-1236, 1986

Fisler, J.S., Bray, G.A. Dietary obesity: A metabolic hypothesis. Obesity: Towards a Molecular Approach, G.A. Bray, D. Riequier and B.M. Spiegelmam eds., UCLA Symposia on molecular and cellular biology, 132: 29-43, Wiley-Liss, Inc., New York, 1990.

Flatt, J.P. Importance of nutrient balance in body weight regulation. Diabetes/Metabolism Rev., 4: No 6, 571-581, 1988

Flatt, J.P., Pahud, P., Ravussin, E., Jéquier, E. An estimate of the P:O ratio in man. Trends in Biochem. Sci., 9: 466-468, 1984
Forbes, G.B. & Reina, J.C, Adult lean body mass declines with age: some longitudinal observations. Metabolism, 19: 653-663, 1970

Fletcher, J.M., Haggarty, P., Wahle, Reeds, P.J. Hormonal studies of young lean and obese Zucker rat. Horm. Metab. Res., 18: 290-295, 1986

Foster, D.O. Participation of alpha-adrenoceptors in brown adipose tissue thermogenesis in vivo. int. J. Obes. 9, suppl., 2: 25-29, 1985

Foster, D.O., Frydman, M.L. Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. Can. J. Physiol. Pharmacol., 57: 257-270, 1979

Foster, D.O., Frydman, M.L. Nonshiverig thermogenesis in the rat. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenalina. Can. J. Physiol. Pharmacol., 56: 110-122, 1978

Garrow, J.S. The regulation of energy expenditure in man. In: Recents Advances in Obesity Research II, G.A. Bray ed., Newman Publishing, London, pp 200-210, 1978

Garza, C., Scrimshaw, N.S., Young, V.R. Human protein requirement: A long-term metabolic nitrogen balance study in young men to evaluate the FAO/WHO safe level of egg protein intake. J. Nutr., 107: 335-352, 1977

Giorgino, R., Cignarelli, M., Garruti, G., De Pergola, G. Obesity and autonomic nervous system. In: Obesity: basic concepts and clinical aspects. Front Diabetes. Belfiore, Jeanrenaud, Papalia eds., 11: 37-49, 1992

Girardier, L., Stock, M.J. Mammalian Thermogenesis. L. Girardier, M.J. Stock, Eds., Chapman and Hall, London, 1983

Godbole, V., York, D.A., Bloxham, D.P. Developmental changes in the fatty (fa/fa) rat: Evidence for defective thermogenesis preceding the hyperlipogenesis and hyperinsulinaemia. Diabetologia, 15: 41-44, 1978

Gordon, C.J. Thermal biology of the laboratory rat. Physiol. Rev., 47: 963-991, 1990

Gruen, R., Hietanen, E., Greenwood, M.R.C. Increased adipose tissue lipoprotein lipase activity during the development of the genetically obese rat (fa/fa). Metabolism, 27: 1955-1966, 1978

Guillaume-Gentil, C., Rohner-Jeanrenaud, F., Abramo, F., Bestetti, G.E., Rossi, G.I., Jeanrenaud, B. Abnormal regulation of the hypothalamo-pituitary-adrenal axis in genetically obese fa/fa rat. Endocrinology, 126: 1873-1879, 1990

Hainault, I., Guere-Millo, M., Guichard, C., Lavau, M. Differential regulation of adipose tissue glucose transporters in genetic obesity (fatty rat): Selective increase in the adipose cell/muscle glucose transporter (GLUT4) expression. J. Clin. Invest., 87: 1127-1131, 1991

Hales, C.N., Kennedy, G.C. Plasma glucose, non-esterified fatty acids and insulin concentrations in hypothalamic-hyperphagia rats. Biochem. J., 90: 620-624, 1964

Harris, R.B. Role of set-point theory in regulation of body weight. FASEB J., 4: 3310-3318, 1990

Hausman, G.J., Kasser, T.R., Shapira, J.F., Martin, R.F. Techniques for identification of young obese Zucker rat and some obsevations on brown adipose tissue morphology and histochemistry in the young obese Zucker rat. Int. J. Obes., 7: 487-492, 1983

Herbai, G. Weight loss in obese-hyperglycemic and normal mice following transauricular hypophysectomy by a modified technique. Acta Endocrinol., 65: 712-722, 1970

Herberg, L., Coleman, D.L. Laboratory animals exhibiting obesity and diabetes syndromes. Metabolism, 26: 59-99, 1977

Herberg, L., Dopper, W., Major, E., Gries, F.A. Dietary induced hypertrophic hyperplastic obesity in mice. J. Lip. Res., 15: 580-585, 1974

Herrero, S. Radio-frequency current and direct current lesions in the ventromedial hypothalamus. Am. J. Physiol. 217: 403- 410, 1969

Himms-Hagen, J. Role of thermogenesis in the regulation of energy balance in relation to obesity. Can. J. Physiol. Pharmacol., 67: 394-401, 1989

Holt, S.J., York, D.A. The effect of adrenalectomy on GDP binding to brown-adipose-tissue mitochondria of obese rats. Biochem. J., 208: 819-822, 1982

Horwitz, B.A. Oubain-sensitive component of brown fat thermogenesis. Am. J. Physiol., 224: 352-355, 1973

Hsieh, A.C.L., Carlson, L.D., Gray, G. Role of the sympathetic nervous system in the control of chemical regulation of heat production. Am J, Physiol., 190: 247-251, 1957

Hustvedt, B.E. & Lovo, A. Correlation between hyperinsulinemia and hyperfagia in rats with ventromedial hypothalamic lesions. Acta Phisiol. Scand., 84: 29-33, 1972

Ingalls, A.M., Dickie, M.M., Snell, G.D. Obese, new mutation in the mouse. J. Hered., 41: 317-318, 1950

Ingbar, S.H., Woeber; K.A. The thyroid gland. En: Williams, R.H. ed. Text-book of endocrinology Saunders, Filadelphia, 117-247, 1981

Inoue, S., Bray, G.A. Sensivity of β-cells to streptozotocin in lean and obese rats. Horm. Metab. Res., 10: 273-280, 1978

James, W.P.T., Trayhurn, P. An integrated view of the metabolic and genetic basis for obesity. Lancet, 2: 770-773, 1976

James, W.P.T., Trayhurn, P. Thermogenesis and obesity. Brit. Med. Bull., 37: No 1, 43-48, 1981

Jansky, L. Non-shivering thermogenesis and its thermoregulatory significance. Biol. Rev., 48: 85-132, 1973

Jeanrenaud, B. An oveview of experimental models of obesity. In: Recent Advances in Obesity Research II, G.A. Bray ed., Newman Publishing, London, pp 111-122, 1978

Jéquier, E., Gygax, P.-H., Pittet, P., Vannotti, A. Increased thermal body insulation: relatioship to the development of obesity. J. Appl. Physiol., 36: 674-678, 1974

Jéquier, E., Acheson, K. , Schutz, Y. Assessment of energy expenditure and fuel utilization in man. Ann. Rev. Nutr., 7: 187-208, 1987

Johnson, P.R., Greenwood, M.R.C., Horwitz, B.A., Stern, J.S. Animals models of obesity: Genetics aspects. Ann. Rev. Nutr., 11:325-353, 1991

Jung, R.T., Shetty, P.S., James, W.P.T. The effect of refeeding after semistarvation on catecholamine and tyroid metabolism. Int. J. Obes., 4: 95-100, 1980

Jung, R.T. Endocrinological aspects of obesity. Clin. Endocrin. Metabol., 13: 597-612, 1984

Kaplan, M.L. Comsumption of O_2 and early detection of fa/fa genotype in rats. Metabolism, 28: 1147-1151, 1979

Karakash, C.,Hustvedt, B.E., Lovo, A., Lemarchand, Y., Jeanrenaud, B. Consequences of ventromedial hypothalamic lesions on metabolism of perfused rat liver. Am. J. Physiol., 232: E286-E293, 1977

Kasser, T.R., Harris, R.B.S., Martin, R.J. Level of saciety: GABA and pentose shunt activities in three brain sites associated with feeding. Am. J. Physiol., 248: R453-R458, 1985

Keesey, R.E., Powley, T.L. The regulation of body weight. Ann. Rev. Psychol. 37: 109-133, 1986

Kennedy, G.C., Parker, R.A. The islets of Langerhansin rats with hypothalamic obesity. Lancet, 2: 981-982, 1963

Kennedy, G.C. The role of depot fat in the hypothalamic control of food intake in the rat. Proc. Royal Soc. B Biol. Sci., 140: 578-592, 1953

Kimura, H., Kuriyama, K., Distribution of gamma-aminobutyric acid (GABA) in the rat hypothalamus: funtional correlates of GABA with activities of appetite controlling mechanisms. J. Neurochem., 24: 903-907, 1975

Kondo (o Konello), K., Nozawa, K., Tomita, T., Ezaki, K. Inbred straints resulting from Japanese mice. Bull. Exp. Anim., 6: 107-112, 1957

Kral, J., Powley, T., Brooks, C. Vagal nerve function: Behaviral and methodological considerations. Nervous System, 9: 1-345, 1983

Krief, S., Bazin, R. Genetic obesity: Is the defect in the sympathetic nervous system ? A review throgh developmental studies in the preobese Zucker rat. Proc. Exp. Biol. Med., 196: 528-538, 1991

Kurzer, M.S., Calloway, D.H. Nitrate and nitrogen balance in men. Am. J. Clin. Nutr., 34: 1305-1313, 1981

Lachance, P.A., Miller, G.A. Protein quality asessement in the rat: correlation between whole carcass and hind limb nitrogen concentration. Nutr. Rep. Int., 7: 25-32, 1973

Lakshmann, F.L., Perera, D.A., Scrimshaw, N.S., Young, V.R. Plasma and urinary amino acids selected sulfur metabolites in young men fed a diet devoid of methionine and cystine. Am. J. Clin. Nutr. 29: 1367, 1976

Langhans, W., Scharrer, E. Evidence for role of the sodium-pump of hepatocytes in the

control of the food. J. Autonom Nerv. Sys, 20: 1199, 1987

Langhans, W., Wiesenreiter, F., Scharrer, E. Different effects of subcutaneous D,L-3-hydroxybutyrate and acetoacetate injections on food intake in rats. Physiol. Behav., 31, 483, 1983

Lavau, M., Bazin,R. Inguinal fat pad weight plotted versus body weight as a method of genotype identification in 16-day-old Zucker rats. J. Lipid Res., 23: 941-943, 1982

Lavau, M., Bazin, R., Guerre-Millo, M. Increased capacity for fatty acid synthesis in white and brown adipose tissues from 7-day-old obese Zucker pups. Int. J. Obes., 9: 61-66, 1985

Lawes, J.B., Gilbert, J.H. On the composition of oxen, sheep and pigs and their increase whilst fattening. J. Royal Agricul. Soc. Engl., 21: 1-92, 1861

Le Magnen, J. Body energy balance and food intake: a neuroendocrine regulatory mechanism. Physiol. Rev., 63: 314-322, 1983

Lean, M.E.J. Brown adipose tissue and obesity. In: Obesity: basic concepts and clinical aspects. Front Diabetes. Belfiore, Jeanrenaud, Papalia eds. 11: 37-49, 1992

Lean, M.E.J. Brown adipose tissue in humans. Proc. Nutr. Soc., 48: 243-256, 1989

Leibowitz, S.F., Brown, O., Tretter, J., Kirschgessner, A. Norepinephrine, clonidine and tricyclicantidepressants selectively stimulate carbohydrate ingestion through noradrenergic system of the paraventricular nucleus. Pharmacol. Biochem. Behav., 23: 541-550, 1985

Leibowitz, S.F., Weiss, G.F., Shor-Posner, G. Medial hypothalamic serotonin in the control of eating behavior. Int. J. Obesity, 11: suppl.3, 109-123, 1987

Levin, B.E., Sullivan, A.C. Catecholamines levels in discrete brain nuclei of seven month old genetically obese rats. Pharmacol. Bichem. Behav., 11: 77-82, 1979

Levine, S.Z., Dann, M., Marpes, E. A defect in metabolism of tyrosine and phenylalanine in premature infants. 3. Demonstration of the irreversible conversion of phenylalanine to tyrosine in the human organism. J. Clin.Invest., 22: 551-562, 1943

Lin, M.H., Romsos, D.R., Akera, T., Leveille, G.A. Na^+,K^+-ATPase enzyme units in skeletal muscle form lean and obese mice. Biochem. Biophys. Res. Commun., 80: 398-404, 1978

Lin, C.P., Huang, P.C. Actual nitrogen deposition in mature adults ratsfed moderate to high protein diets. J. Nutr., 112: 1067-1074, 1982

Lin, P.Y., Romsos, D.R., Vander Tuig, J.G., Lveille, G.A. Maintenanceenergy requirements, energy retention and heat production of young obese (ob/ob) and lean mice fed a high-fat or high-carbohydrate diet. J. Nutr., 109: 1143-1153, 1979

Loten, E., Le Marchand, Y., Assimacopoulous, F. et al. Does hyperinsulinemia in ob/ob mice cause an insuline stimulated adipose tissue. Am. J. Physiol., 230: 602-607, 1976

Luiten, P.G.M., ter Horst, G.J., Steffens, A.B. The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. Prog. Neurobiol., 28: 1-54, 1987

Lloyd, L., McDonald, B.E., Crampton, E.W. Fundamentals of Nutrition. Ed. G.W. Salisbury, Freeman and Company, San Francisco. Second edition, 1978

Ma, S.W.Y., Foster, D.O. Brown adipose tissue, liver, and diet-induced thermogenesis in cafeteria diet-fed rats. Can. J. Physiol. Pharmacol., 67: 376-381, 1989

Malewiak, M.I., Grglio, S., Le Liepvre, X. Relationship between lipogenesis, Ketogenesis and malonyl-coA content in isolated hepatocytes from the obese Zucker rat adapted to high-fat diet. Metabolism, 34: 604-611, 1985

Marchington, D., Rothweel, N.J., Stock, M.J., York, D.A. Energy balance, diet-induced thermogenesis and brown adipose tissue in lean and obese (fa/fa) Zucker rats after adrenalectomy. J. Nutr., 113: 1395-1402, 1983

Marchington, D., Rothwell, N.J., Stock, M.J., York, D.A. Thermogenesis and sympathetic activity in brown adipose tissue of overfed rats following adrenalectomy. Am. J. Physiol., 250: E362-366, 1986

Martin R.J., Wangsness, P.J., Gahagan, J.H. Diurnal changes in serum metabolites and hormones in lean and obese Zucker rats. Horm. Metab. Res., 10: 187-192, 1978

Martin, R.J., Drewry, M., Jewell, D., Harris, R.B.S., Young, R., Patton, J.S. Grwowth hormone treatment reduces total body fat accumulation in Zucker obese rats. Int. J. Obes., 13: 327-335, 1989

Martin, R.C., Gahagan, J.H. The influence of age and fasting on serum hormones in lean and obese Zucker rats. Proc. Soc. Exp. Biol. Med., 154: 610-614, 1977

Mayer, J. Glucostatic mechanism of regulation of food intake. New Engl. J. Med., 249: 13-16, 1953

Mayer, J. Regulation of enrgy intake and body weight: the glucostatic theory and lipostatic hypothesis. Ann. N.Y. Acad. Sci., 63: 15-43, 1955

Mayer, J., French, R.G., Zighera, C.F, Barnett, R.J. Hypothalamic obesity in the mouse: production, description and metabolic characteristics. Am. J. Physiol., 182: 75-82, 1955

McGinty, D., Epstein, A.N., Teiltelbaum, P. The contribution of oropharyngeal sensations to hypothalamic hyperphagia. Animal Behav., 13: 413-418, 1965

McKeown, T., Record, R.G. The influence of reproduction on body weight in women. J. Endocrinol., 15: 393-409, 1957

McLaughlin, C.L., Baile, C.A., Della-Fera, M.A., Kasser, T.G. Meal-stimulated increased concentrations of CCK in the hypothalamus of Zucker obese and lean rats. Physiol. Behav., 35: 215-220, 1985

Meguid, M.M., Matthews, D.E., Bier, D.M, Meredith, C.N., Soeldner, J.S., Young, V.R. Leucine kinetics at graded leucine intakes in young men. Am. J. Clin. Nutr., 43: 770-778, 1986

Meister, A. Biochemistry of the amino acids. 2 Ed., Academyc Press, New York, 1965

Mejsnar, J., Jansky, L. Methods for estimating non-shivering thermogenesis. In: Non-shivering thermogenesis. Jansky eds., 27-36, 1971

Mendel, L.B. Nutrition and growth. Harvey Lectures, 10: 101-131, 1914-1915

Merril, A.L., Watt, B.K. Energy values of food. In: Agricultural Handbook 74. Washington, 1955

Modan, M., Halkin, H., Almog, S. et al. Hyperinsulinemia: a link between hypertension, obesity and glucose intolerance. J. Clin. Invest., 75: 809-817, 1985

Mook, D.G., Fisher, J.C., Durr, J.C. Some endocrine influences on hypothalamic hyperphagia. Horm. Behav., 6: 65-79, 1975

Moore, B.J. The cafeteria diet -an inappropiate tool for studies of thermogenesis. J. Nutr., 115: 1447-1458, 1985

Morley, J. Neuropeptide regulation of appetite and weight. Endocrine Rev., 8: 256-287, 1987

Mount, L.E. Heat transfer between animal and environment. Proc. Nutr. Soc., 37: 5-12, 1978

Munro, H.N., Crim, M. Modern nutrition in health and disease. In: Shils, M.E., Goodhart, R.S., eds. 6th. ed., Philadelphia: Lea & Febiger, 51-55, 1980

Nacht, C.A., Christin, L., Temler, E., Chiolero, R., Jequier, E., Acheson, K.J. Thermic effect of food: possible implication of parasympathetic nervous system. Am. J. Physiol., 253: E481-E488, 1987

Nakamura, M., Yamada, K. A further study of the diabetic (KK) strain of the mouse. F_1 and F_2 offsprings of the cross between KK and C57Bl mice. Proc. Japan Acad., 39: 489-493, 1963

Nicholls, D.G. Brown adipose tissue mitochondria. Biochim. Biophys. Acta., 549: 1-29, 1979

Nishizawa, Y., Bray, G.A. Evidence of circuling ergostatic factor: Studies on parabiotic rats. Am. J. Physiol. 239: R344-350, 1980

Nuñez, A.A., Grundman, M. Testosterone affects food intake and body weight of weanling male rats. Pharm. Biochem. Behav., 16: 933-936, 1982

Ohshima, K., Shargill, N.S., Chan, T.M., Bray, G.A. Adrenalectomy reverses insulin resistance in muscle from obese (ob/ob) mice. Am. J. Physiol., 246: E193-E197, 1984

Ohshima, K., Shargill, N.S., Chan, T.M., Bray, G.A. Adrenalectomy reverses insulin resistance in muscle from obese (ob/ob) mice. Am. J. Physiol., 246: E193-197, 1984

Okada, S., Bray, G., York, D., Erlanson-Albertsson, C. VPDPR-A natural peptide which suppresses fat intake in Osborne-Mendel rats. Int. J. Obes., 14 (suppl. 12): 54, 1990

Olefsky, J.M. Insulin resistance and insulin action: an in vitro and in vivo perspective. Diabetes, 30: 148-162, 1981

Osborne, T.B., Mendel, L.B. Amino acids in nutrition and growth. J. Biol. Chem., 17: 325-349, 1914

Pastor-Anglada, M., Remesar, X. Urea and ammonia in urine and plasma of fed-mit-pregnant rats. Biol. Res. Pregnancy, 7: 134-137, 1986

Pastor-Anglada, M., Remesar, X. Urinary amino acid excretion in pregnant rat. Nutr. Res., 6: 709-718, 1986

Paul, A.A., Southgate, D.A. In: The composition of foods. McCance & Widdowson, London, 1978

Paul, A.A., Southgate, D.A. In: The composition of foods. McCance & Widdowson, London, 1978

Payne, P.R., Dugdale, A.E. Mechanisms for the control of body weight. Lancet, I: 583-586, 1977

Planche, E., Joliff, M., deGasquet, P., Le Liepvre, X. Evidence of defect in energy expenditure in a 7-day-old Zucker rat (fa/fa). Am. J. Physiol., 245: E107-E113, 1983

Poe, R.H., Davis, T.R.A. Cold exposure and acclimation in alloxan-diabetic rats. Am. J. Physiol., 202: 1045-1048, 1962

Poole, S., Stephenson, J.D. Body temperature regulation and thermoneutrality in rats. Q. J. Exp. Physiol., 62: 143-149, 1977

Postel-Vinay, M.C., Durand, D., Lopez, S., Kayser, C., Lavau, M. Increased growth hormone binding to liver membranes of obese Zucker rats. Horm. Metab. Res., 22: 7-11, 1990

Powers, P.S. Obesity. The regulation of weight. Baltimore: Williams & Wilkins, 1980

Powley, T.L. The ventromedial hypothalamic obesity abolished by subdiaphragmatic vagotomy. Am. J. Physiol., 226: 25-33, 1974

Prats, E., Monfar, M., Castellà, J., Iglesias, R., Alemany, M. Energy intake of rats fed a cafeteria diet. Physiol. Behav., 45(2): 263-272, 1989

Rabin, B.M. Independence of food intake and obesity following ventromedial hypothalamic lesions in the rat. Physiol. Behav., 13: 769-772, 1974

Radcliffe, J.D., Webster, J.F. Sex, body composition and regulation of food intake during growth in the Zucker rat. Br. J. Nutr., 39: 483-492, 1978

Rebuffé-Scrive, M. Steroid hormones and distribution of adipose tissue. Acta Med. Scand suppl., 723: 143-150, 1987

Rand, W.M., Scrimshaw, N.S., Young, V.R. Determination of protein allowances in human adults from nitrogen balance data. Am. J. Clin. Nutr. 30: 1129-1134, 1977

Rebuffé-Scrive, M., Anderson, B., Olbe, L. et al. Metabolism of adipose tissue in intraabdominal depots in non-obese men and women. Metabolism, 38: 453-467, 1989

Reeds, P.J., Haggarty, P., Wahle, K.W.J., Fletcher, J.M. Tissue and whole-body protein synthesis in immature Zucker rats and their relationship to protein deposition. Biochem. J., 204: 393-398, 1982

Remesar, X., Arola, Ll., Palou, A., Alemany, M. Body and organ size and composition during the breeding cycle of rats (Rattus norvegicus). Laboratory Anim. Sci., 31: 67-70, 1981

Rogers, P.J. Returning "cafeteria-fed" rats to a chow diet: negative contrast and effects of obesity on feeding behaviour. Physiol. Behav., 35: 493-499, 1985

Rogers, A.E. Nutrition. In: The laboratory rat. Baker, H.J., Lindsey, J.R., Weisbroth, S.H. eds., 1: 123-152, New York: Academic Press, 1979

Rognstad, R. Sources of ammonemia for urea synthesis in isolated rat liver cells. Biochem. Biophys. Acta, 496: 249-259, 1977

Rohner-Jeanrenaud, F., Jeanrenaud, B. Involvement of the cholinergic system to insulin and glucagon oversecretion of genetic preobesity. Endocrinology, 116: 830-834, 1985

Rohner-Jeanrenaud, F., Hochstrasser, A.C.,Jeanrenaud, B. Hyperinsulinemia of preobese and obese fa/fa rats is partly vagus nerve mediated. Am. J. Physiol., 244: E317-E322, 1983

Rolls, B.J., Rowe, E.A, Turner, R.C. Persistent obesity in rats following a period of consumption of a mixed, high energy diet. J. Physiol., 298: 415-427, 1980

Rose, W.C., Wixom, R.L. The amino acids requirements of man. XVI The role of nitrogen intake. J. Biol. Chem., 217: 997-1004, 1955

Rothwell, N.J., Stock, M.J. Thermogenesis and brown adipose tissue activity in hypophysectomised rats with and without corticotropin replacement. Am. J. Physiol., 249: E333-336, 1985

Rothwell, N.J., Stock, M.J. Sympathetic and adrenocortidoid influences on diet-induced thermogenesis and brown fat activity in the rat. Comp. Biochem. Physiol., 79A: 575-579, 1984

Rothwell, N.J., Stock, M.J. Influences of adrenalectomy on age-related changes in energy

balance, thermogenesis and brown fat activity in the rat. Comp. Biochem. Physiol., 89A: 265-269, 1988

Rothwell, N.J., Stock, M.J. Effects of feeding a palatable "cafeteria" diet on energy balance in young and adult lean (+/?) Zucker rats. Br. J. Nutr., 47: 461-471, 1982

Rothwell, N.J., Stock, M.J. Similarities between cold- and diet-induced thermogenesis in the rat. Can. J. Physiol. Pharmacol., 58: 842-848, 1980

Rothwell, N.J., Stock, M.J. Regulation of energy balance. Ann. Rev. Nutr., 1: 235-256, 1981

Rothwell, N.J., Stock, M.J. Thermogenesis induced by cafeteria feeding in young growing rats. Proc. Nutr. Soc., 39: 5A, 1980

Rothwell, N.J., Stock, M.J. Effects of feeding a palatable cafeteria diet on energy balance in young and adult lean (+/?) Zucker rats. Br. J. Nutr., 47: 461-471, 1982

Rothwell, N.J., Stock, M.J. A role for brown adipose tissue in diet-induced thermogenesis. Nature, 281: 31-35, 1979

Rothwell, N.J., Stock, M.J. Intra-strain difference in the response to overfeeding in the rat. Proc. Nutr. Soc., 39: 5A, 1980

Saito, M., Bray, G.A. Adrenalectomy and food restriction in the genetically obese (ob/ob) mice. Am. J. Physiol., 246: R20-R25, 1984

Salans, L.B. The obesities. In: P.Feling, J.D. Baxter,A.E Broadus and L.A. Frohman eds. Endorinology and Metabolism, New York: McGraw-Hill, 891-916, 1981

Sarwar, G., Peace, R.W., Botting, H.G. Validity of rat plasma amino acids in predicting first limiting amino acids in protein mixtures. Nutr. Rep. Int., 28: 613-620, 1983

Saul, R.L., Archer, M.C. Oxidation of ammonia and hydroxylamine to nitrate. Carcinogenesis, 5: 77-81, 1984

Sclafani, A., Aravich, P., Landman, M. Vagotomy blocks hypothalamic hyperphagia in rats on a chow diet and sucrose solution, not on a mixed palatable diet. J. Comp. Physiol., 244: R686-R694, 1981

Sclafani, A., Berner, C.N. Hyperphagia and obesity produced by parasagittal and coronal hypothalmic knife cuts-further evidence for a longitudinal feeding inhibitory pathway. J. Comp. Physiol., 91: 1000-1018, 1977

Sclafani, A., Springer, D. Dietary obesity in adult rats, similarities to hypothalamic and human obesty syndromes. Physiol. Behav., 17: 461-471, 1976

Scriver, C.R., Rosenberg, L.E. Nutritional aspects of amino acid metabolism. In: Amino acid matebolism and disorders. Saunders, Philadelphia-London-Toronto, 1973

Schemmel, R.A., Teague, R.J., Bray, G.A. Obesity in Osborne-Mendel and S5B/Pl rats:

effects of sucrose solution, castration, and teatment with estradiol or insulin. Am J. Physiol., 243: R347-R353, 1982

Schemmel, R.A., Mickelsen, O. Influence of diet, strain, age and sexe on fat depot mass and body composition of the nutritionally obese rat. In: The regulation of Adipose Tissue Mass. J. Vague, J. Boyer eds., American Elsevier, New York, 238-253, 1974

Schirardin, H., Bach, A., Schaeffer, A., Baeur, M., Weryha, A. Biological pararmeters of the blood in the genetically obese Zucker rat. Arch. Int. Physiol. Biochim., 87: 275-289, 1979

Schnatz, J.D., Bernardis, L.L., Frohman, L.A., Goldman, J.K. Hypertriglyceridemia in weanling rats with hypothalamic obesity. Diabetes, 20: 655-663, 1971

Schonbaum, E., Johnson, G.E., Sellers, E.A., Gill, M.J. Adrenergic beta-receptors and non-shivering thermogenesis. Nature, 210: 426-429, 1966

Seydoux, J. Défaut de thermogenèse et obesité. Médecine/Sciences, 3: 387-393, 1987

Sharrer, E., Langhans, W. Control of food intake by fatty acid oxidation. Am. J. Physiol., 250: R1003-R1006, 1986

Shibata, H., Perusse, F., Bukowiecki, L.J. The role of insulin in non-shivering thermogenesis. Can. J. Physiol. Pharmacol., 65: 152-158, 1987

Shimazu, T. Central nervous system regulation of fat metabolism. En: Hales, J.R.S. ed. Thermal Physiology, New york: Raven Press, 183-188, 1984

Silva, J.E., Larsen, P.R. Potencial of brown adipose tissue type II thyroxine 5'deiodinase as a local and systemic source of triiodothyronine in rats. J. Clin. invest., 76: 2296-2305, 1985

Sims, E.A.H., Horton, E.S. Endocrine and metabolic adaptation to obesity and starvation. Am. J. Clin. Nutr., 21: 1455-1470, 1968

Sims, E.A.H., Danforth, E., Horton, E.S., Bray, G., Glennon, J.A., Salans, L.B. Recent Prog. Horm. Res., 30: 457-496, 1973

Sinha, Y.N., Salocks, C.B., Vanderlaan, W.P. Prolactin and growth hormone secretion in chemically induced and genetically obese mice. Endocrinology, 97: 1386-1393, 1975

Smith, R.E., Horwitz, B.A. Brown fat and thermogenesis. Physiol. Rev., 49: 330-425, 1969

Smith, P.E. The disabilities caused by hypophysectomy and their repair. J. Am. Med. Assoc., 88: 158, 1927

Smith, T.J., Edelman, I.S. The role of sodium transport in thyroid thermogenesis. Federation Proc., 38: 2150-2153, 1979

Snowdon, C.T. Motivation, regulation and the control of meal parameters with oral and

intragastric feeding. J. Comp. Phychol., 69: 91-100, 1969

Snyderman, S.E., Amino acid requirements. History of Pediatrics 1850-1950, Edited by B.L. Nichols, A. Ballabriga, N. Kretchmer. Nestlé Nutrition Workshop Series. Vol 22: 211-218. Nestec Ltd., Vevey/Raven Pres, Ltd., New York, 1991

Sotelo-López, A., Lucas- Florentino, B. Determination of net protein utilization using whole carcass, hind leg or liver of the rat and its relations with protein efficiency ratio determination. J. Nutr., 108: 61-66, 1978

Southgate, D.A.T., Durnin, J.V.G.A. Caloric conversion factors. An experimental re-assessment of the factors used in the calculation of the energy value of human diets. British J. Nutr., 24: 517-535, 1970

Stanley, B.G., Daniel, D., Chin, A., Leibowitz, S. Paraventricular nucleus injections of Peptide YY and Neuropeptide Y preferentially enhance carbohydrate ingestion. Peptides, 6: 1205-1211, 1985

Stock, M., Rothwell, N. Obesity and leanness. Basic aspects. London: John Libbey, 76-87, 1982

Stunkard, A.J., Sorensen, T.A., Hanis, C., Teasdale, T.W., Chakraborty, R., Schull, W.J., Schulsinger, F. An adoption study of human obesity. N. Engl. J. Med., 314: 193-199, 1986

Stunkard, A.J., Foch, T.T., Hrubec, Z. A twin study of human obesity. J. Am. Med. Assoc., 256: 51-54, 1986

Tarui, S., Fujiota, S., Tokunaga, K., Matsuzawa, Y. Comparison of pathophysiology betwen subcutaneous-type and visceral-type obesity. Bray,G.A., LeBlanc, J., Inoue, S. and Suzuki, M. eds. Japan Science Societies Press, Tokyo/S. Karger, Basel, 143-152, 1988

Terretaz, J., Assimacopoulus-Jeannet, F., Jeanrenaud, B. Severe hepatic and perpheral insulin resistance as evidenced by euglycemic clamps genetically obese fa/fa rats. Endocrinology, 118: 674-678, 1986

Tilve, S.G. Variations in daily urinary-N of adult rats on varying protein levels. J. Nutr., 112: 453-460, 1982

Trayhurn, P., James, W.P.T. Thermogenesis: Dietary and non-shivering aspects. In: The body weight regulatory system: Normal and disturbed mechanisms. Cioffi, L.A., James, W.P.T., Van Italle, T.B. eds., Raven Press, New York, 1981

Trayhurn, P., James, W.P.T. Thermogenesis and obesity. In: Girardier I., Stock M.J., eds. Mammalian thermogenesis, London: Chapman & Hall, 234-258, 1983

Triandafillou, J., Himms-Hagen, J. Normal cold- but defective diet-induced activation of brown adipose tissue mitochondria in genetically obese (fa/fa) rats. Am. J. Physiol., 244: E145-E150, 1983

Triscari, J., Greenwood, M.R.C., Sullivan, A.C. Oxidation and Ketogenesis in the

hepatocytes of lean and obese Zucker rats. Metabolism, 31: 223-228, 1982

Truett, G.E., Bahary, N., Friedman, J.M., Leibel,R.L. Rat obesity gene fatty (fa) maps to chromosome 5: Evidence for homology with the mouse gene diabetes (db). Proc. Natl. Acad. Sci., 88: 7806-7809, 1991

Vallerand, A.L., Lupien, J., Bukowiecki, L.J. Interaction of cold-exposure and starvation on glucose tolerance and insulin response. Am. J. Physiol., 245: 227-232, 1983

Van Itallie, T.B. The glucostatic theory 1955-1988: Roots and branches. Int. J. Obes., 14: 1-10, 1990

Vasselli, J.R., Cleary, M.P. and VanItallie T.B. Modern concepts of obesity. Nutr. Rev., 41: 361-373, 1983

Vaughan, R.W., Conahan, T.J. Cardiopulmonary consequences of morbid obesity. Life Sci., 26: 2119-2127, 1976

Viñas, O., Vilaró, S., Herrera, E., Remesar, X. Effects of chronic ethanol treatement on amino acid uptake and enzyme activities in the lactating rat mammary gland. Life Science, 40: 1745-1749, 1987

Von, L.J. Animal chemistry in its applications to physiology and pathology. (trans. W. Gregory). London: Taylor and Walton, 1842.

Wagner, D.A., Moldawer, L.L., Pomposelli, J.J., Tannenbaum, S.R., Young, V.R. Nitrate biosynthesis in the rat. Biochem. J., 232: 547-551, 1985

Wannemacher, R.W., Dinterman, R.E. Diurnal response in endogenus amino acid oxidation of meal-fed rats. Biochem. J., 190: 663-671, 1980

Waterlow, J.C., Garlick, P.J., Millward, D.J. Protein turnover in mammalian tissues and in the whole body. Amsterdam: Elsevier/North Holland, 1978
Weiner, J.S. The natural history of man. New York: Anchor Press/Doubleday, 143-197, 1973

Weitze, M. Heredytary adiposity in mice and the cause of this anomaly. Copenhagen, Denmark: Univ. of Copenhagen, p 96, 1940

Wiersinga, W.M., Modderman, P. Touber, J.L. The effect of α- and β-adrenoceptor agonists and antagonists on the in vitro conversion of thyroxine into triiodothyronine. Horm. Res., 12: 346-347, 1980

Wilson, P.N., Osbourn, D.R. Compensatory growth after undernutrition in animals and birds. Biol. Rev. Cambridge Philos. Soc., 35:324- 363, 1960

Willcoock, E.G., Hopkins, F.G. The importance of individual amino acids in metabolism. Observations on the effect of adding tryotophan to a dietary in which zein is the sole nitrogenous constituent. J. Physiol (London) 35: 88-102, 1906

Wirtshafter, D., Davis, J.D. Set points, settling points and the control of body weight.

Physiol. Behav., 19: 75-78, 1977

Wisén, O., Rössner, S., Johansason, C. Gastric secretion in massive obesity. Evidence for abnormal response to vagal stimulation. Digest. Dis. Sci., 32: 968-972, 1987

Wolff, G.L. Body compsition and the coat color correlation in different phenotypes of viable yellow mice. Science, 147: 1145-1147, 1965

Wollaston, W.H. On cystic oxide, a new species of urinary calculus. Phil. Trans. B., 100: 223-230, 1810

Wu, S.Y., Stern J.S., Fisher, D.A., Glick, Z. Cold-induced increase in brown fat thyroxin 5'-monodeiodinase is attenuated in Zucker obese rat. Am. J. Physiol., 252: E63-E67, 1987

Yamashita, S., Melmed, S. Insulin regulation of rat growth hormone gen transcription. J. Clin. Invest., 78: 1008-1014, 1986

York, D.A., Bray, G.A., Yukimura, Y. An enzimatic defect in the obese (ob/ob) mouse: Loss of thyroid-induced sodium - and potassium-dependent adenosinetriphosphatase. Proc. Natl. Acad. Sci. USA, 75: 477-481, 1978

York, D.A., Godbole, V. Effects of adrenalectomy on obese "fatty" rats. Horm. Metab. Res., 11: 646-650, 1979

York, D.A., Bray, G.A. Dependence of hypothalamic obesity on insulin, the pituitary and the adrenal gland. Endocrinology, 90: 885-894, 1972

Young, V.R., Hussein, M.A., Murray, E., Scrimshaw, N.S. Plasma tryptophan respose curve and its relation to tryptophan requirements in young adult men. J. Nutr., 101: 45-59, 1971

Young, V.R., Munro, H.N. N-methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. Fed. Proc., 37: 2291-2300, 1978

Young, V.R., Bier, D.M. Amino acid requirements in the adult human: How well do we know them?. J. Nutr., 117: 1484-1487, 1987

Young, J.B., Saville, E., Rothwell, N.J., Stock, M.J., Landsberg, L. Effect of diet and cold exposure on norepinephrine turnover in brown adipose tissue in the rat. J. Clin. Invest., 69: 1061-1071, 1982

Young, V.R. 1987 McCollum Award Lecture. Kinetics of human amino acid metabolism: nutritional implications and some lessons. Am. J. Clin. Nutr., 46: 709-725, 1987

Yukimura, Y., Bray, G.A. Effects of adrenalectomy on thyroid function and insuline levels in obese (ob/ob) mice. Proc. Soc. Biol. Med., 159: 364-367, 1978

Yukimura,Y., Bray, D.A., Wolfsen, A.R. Some effects of adrenalectomy in the fatty rat. Endocrinology, 103: 1924-1928, 1978

Zucker, L.M. Hereditary obesity in the rat associated with hyperlipidemia. Ann. NY Acad

Sci, 131: 447-458, 1965

Zucker, L.M., Efficiency of energy utilization by the Zucker hereditarily obese rat "fatty". Proc. Soc. Exp. Biol. Med., 148: 498-500, 1975

Zucker, T.F., Zucker, L.M. Fatty, a new mutation in the rat. J. Hered., 52: 275-278, 1961

UNIVERSITAT DE BARCELONA

Divisió de Ciències Experimentals
i Matemàtiques

Facultat de Biologia