

Plasma Amino Acids in Hyperphagic Pups Subjected to a Glucose Gavage

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An oral gavage of either glucose or saline was given to pups fed either standard diet or cafeteria diet. The plasma amino acid concentrations were measured by a radiochemical method. In the standard diet group, plasma Asn+Asp, Thr, Pro, Cit, Trp and Phe levels were higher in rats receiving a glucose solution than in those given saline solution; taurine (on day 20) and Ser (on day 30) showed also higher plasma values. Plasma Arg and taurine levels in rats receiving glucose were lower than those in rats receiving saline when these pups were fed the cafeteria diet. Tyr (on day 20) and Gly and Pro (on day 30), showed decreased plasma values. The diet consumed during the days preceding a glucose gavage may have pronounced effects on several metabolites, particularly on nitrogen metabolism. The homeostasis of plasma amino acids was held highly constant in spite of the variety of diets supplied, indicating a remarkable homeostatic capacity on amino acidemia against dietetic manipulation.

Key words: Glucose gavage, Cafeteria diet, Young rats, Amino acids.

A cafeteria diet has been found to induce changes on plasma amino acid concentrations in adult (7, 11, 19, 25) and in young rats (23). Feeding rats with a cafeteria diet has been extensively used as an

experimental model to study obesity and regulation of energy balance. During postnatal development, rats whose dams received cafeteria diets grew more rapidly, and attained a higher degree of maturation than their control-fed counterparts (22). Juvenile rats allocate a larger percentage of their energy intake to the accumulation of lean body mass, whereas adult rats allocate a greater percentage of their energy intake to maintenance and accumulation

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of body fat (14, 27). The rats fed a cafeteria diet show altered nitrogen metabolism, with higher carcass nitrogen retention (6), and diminished activity of the urea cycle (5). The overall result is a higher efficiency of dietary protein nitrogen utilization, since amino acids are used as energy fuels in a low proportion (12), and the availability of essential amino acids for protein synthesis may be enhanced because cafeteria diet resulted in a higher intestinal absorption of amino acids, although this diet had a similar amino acid composition to that of the reference diet (9).

Cafeteria diets have been formulated in a wide range of compositions and administration patterns. Nevertheless, the nutrient composition of the food is remarkably constant (18) in spite of a highly variable selection. The cafeteria diet is grossly hyperlipidic, with lower proportions (but not absolute amounts) of carbohydrate and protein with respect to standard common pellet chow diet (18). Cafeteria-fed rats preferentially metabolize lipids (21) rather than either carbohydrate (10, 21) or protein (4, 17, 24). This is reflected in the plasma levels of metabolites. The aim of this study was to determine the effect of a glucose gavage on plasma amino acid levels in cafeteria diet-fed and in standard chow diet-fed pups.

Materials and Methods

Primiparous Wistar rats were used. They were housed with their litter in individual plastic-bottomed cages with wood shavings as absorbing material. The rats were kept under standard conditions (21–22 °C, light on from 8 to 20 h) in a controlled animal room. Two dietary treatments were given to dams from the moment of delivery: either standard diet, with pellet (type A04 from Panlab, Barcelona) or cafeteria diet, which consists of an excess of: chow pellet, banana, bacon,

cookies, liver paté, chocolate, hazelnuts, soft candy, pastry, and whole milk complemented with 250 g/l of sucrose plus a mineral and vitamin supplement (Gevral, Cyanamid Iberica, Madrid, Spain). All animals had free access to tap water. All litters were equalized to 8 pups after delivery and kept with their mothers until death. The final experimental groups were selected from different litters. From 11 days after birth, pups were given a daily gavage by stomach cannula, at a dose of 10 ml/kg b. w. The gavage consisted of: a) 9 g/l NaCl (saline) in Tween 90 solution; or b) 150 g/l glucose in Tween 90. According to the different treatments, four groups of five pups were studied: 1) saline gavage-standard diet; 2) saline gavage-cafeteria diet; 3) glucose gavage standard diet; 4) glucose gavage-cafeteria diet.

On postnatal day 14, 20 or 30 pups were killed by decapitation. Blood samples were taken in heparinized dry beakers. Plasma was obtained by centrifugation, and small aliquots were deproteinized with cold acetone (2). The clear supernatants were used for amino acid determination with a radiochemical method, based on the specific reaction of amino acids with ¹⁴C-dansyl-chloride and subsequent chromatographic separation and measurement of radioactivity (3). Results for glutamate plus glutamine, aspartate plus asparagine and leucine plus isoleucine, are given as composite values, due to methodological considerations.

Statistical comparisons between groups were performed with three- or two-factor analysis of the variance (ANOVA) tests (8). A $p < 0.05$ limit of significance was used throughout.

Results

Figure 1 shows the concentrations of some individual gluconeogenic and branched chain amino acids in plasma of young rats fed a cafeteria diet or a stan-

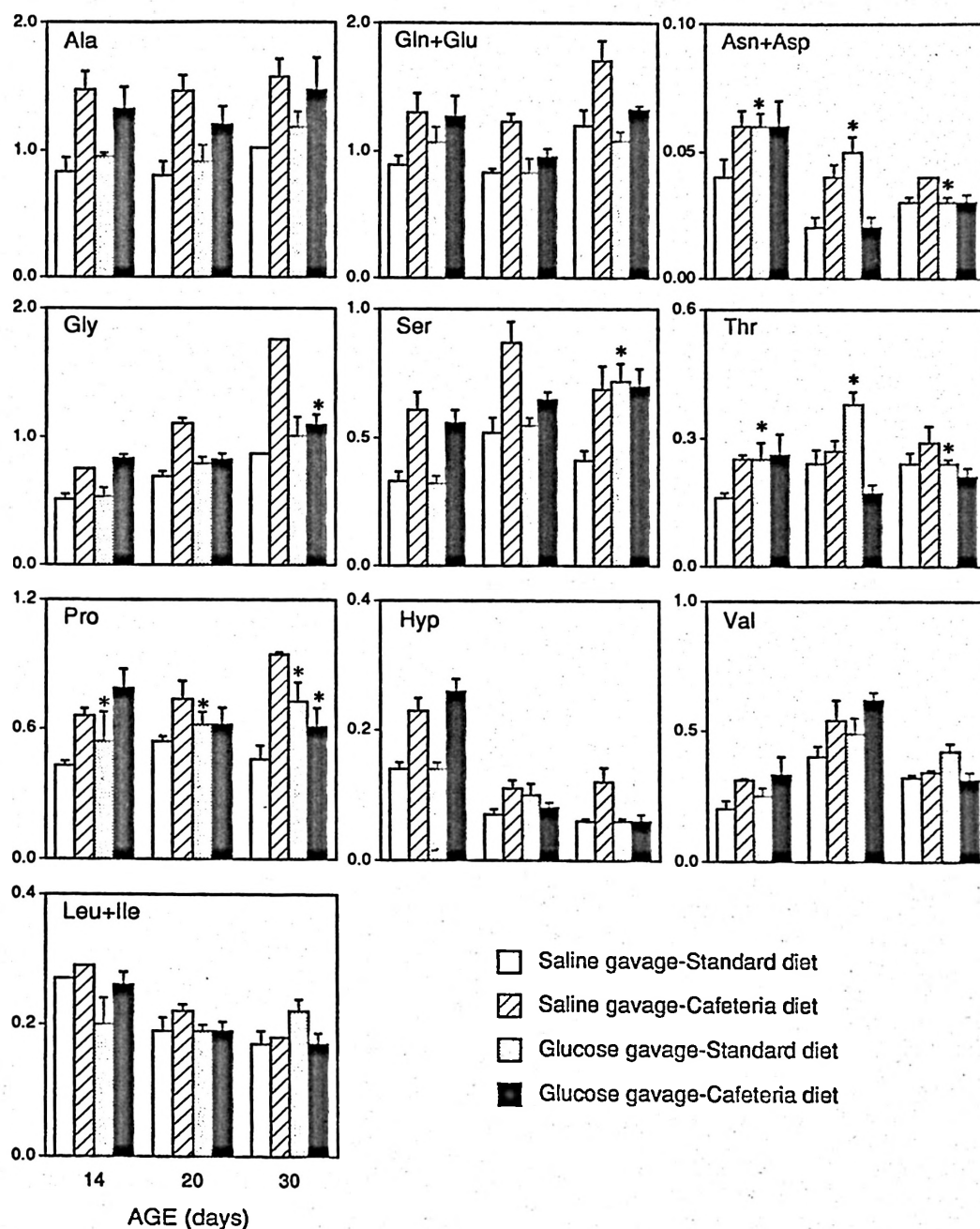


Fig. 1. Individual gluconeogenic and branched-chain free amino acid concentrations in plasma of cafeteria diet-fed or standard diet-fed and glucose gavaged or saline gavaged rats. Ordinate: concentrations expressed as mmol/l. Abscissa: days of age. All values are the mean $\pm$ SEM of 5 animals. Values with * are significantly different for gavage ($p < 0.05$).

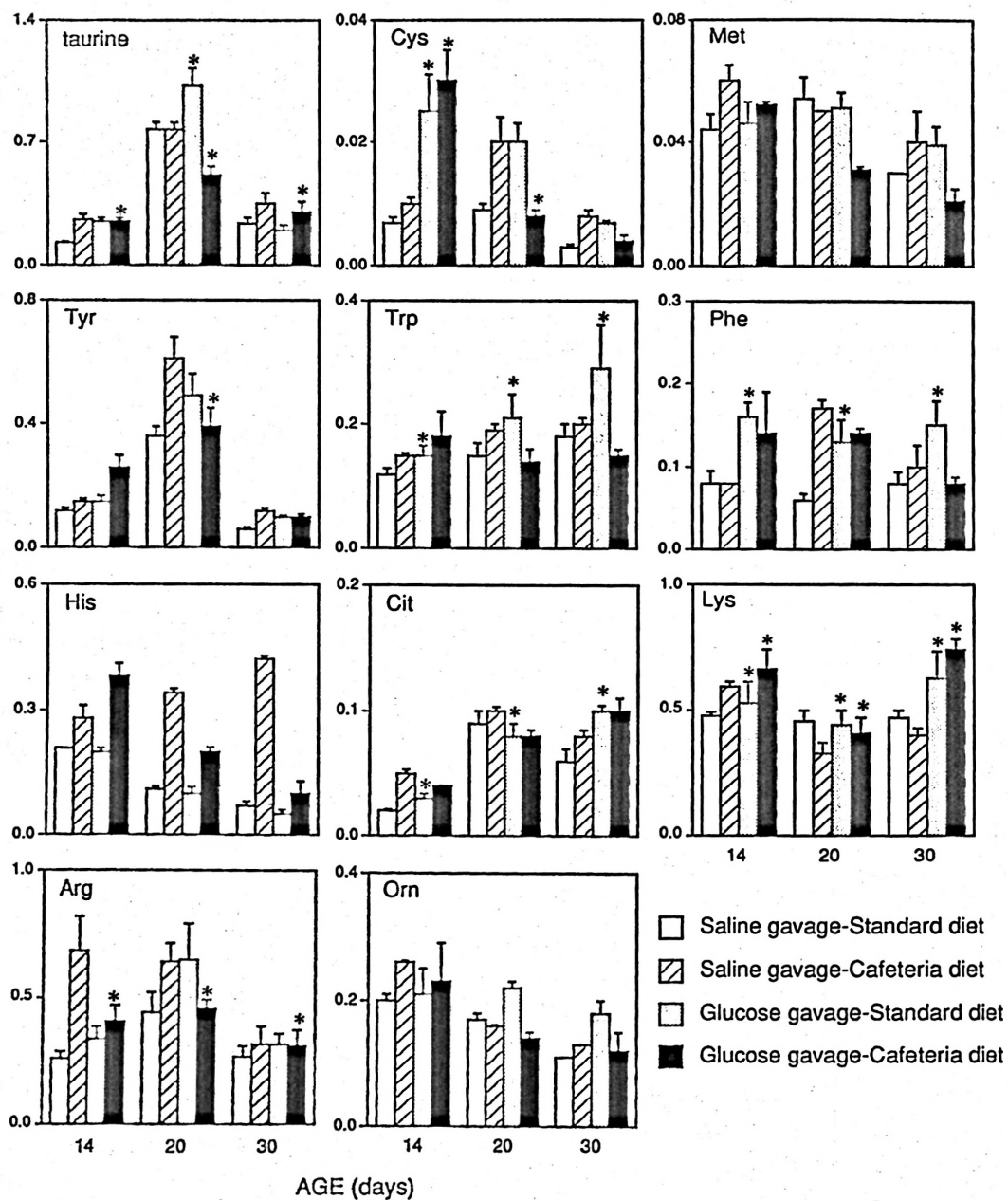


Fig. 2. Individual sulfur, aromatic and basic free amino acid concentrations in plasma of cafeteria diet-fed or standard diet-fed and glucose gavaged or saline gavaged rats. Ordinate: concentrations expressed as mmol/l. Abscissa: days of age. All values are the mean $\pm$ SEM of 5 animals. Values with * are significantly different for gavage ($p < 0.05$).

dard diet, and supplied with a glucose solution or a saline solution. Figure 2 shows the individual variations in aromatic, sulphur and basic amino acids in plasma for the four groups.

Met, Orn and Leu+Ile showed decreasing plasma values with age independently of the treatment used. The changes with age shown by Cit were dependent on gavage, and those of Asn+Asp, Hyp, Arg, Taurine and Tyr were dependent on diet and gavage. Ala and Phe did not change with age during the period studied.

Ala and Gln+Glu were higher in rats fed cafeteria diet than in rats fed standard diet, irrespective of age and gavage used. Other amino acid levels increased with cafeteria diet, but this increase was dependent on gavage (Pro), on age (Val, His), or on gavage and age (Gly, Ser).

Lys was the only amino acid that showed higher levels for the two diets used when the glucose gavage was administered. After 20 days, Lys values decreased in relation to days 14 and 30.

Plasma Asn+Asp, Thr, Pro, Cit, Trp and Phe levels in rats given glucose were higher than those of rats given saline when these pups were fed the standard diet. Moreover, taurine (on day 20) and Ser (on day 30) showed increased plasma values with this treatment. The plasma Cys levels in rats fed the standard diet were higher in glucose than in saline supplied (statistically significant on day 14) rats. In cafeteria diet fed rats when the pups were given glucose solution the plasma concentrations of Cys were higher on day 14, lower on day 20 and the same on day 30 than Cys levels of their counterparts receiving saline solution.

Plasma Arg and taurine levels of rats given glucose were lower than those of rats given saline when these pups were feeding cafeteria diet. Tyr (on day 20) and Gly and Pro (on day 30), also showed lower plasma values with glucose gavage.

Discussion

Cafeteria diet induces a generalized increase in the plasma levels of gluconeogenic amino acids. In the case of branched chain amino acids, plasma Val increases whereas Leu+Ile is not affected. All essential amino acids decreased in cafeteria diet fed rats as previously observed (23). Branched chain amino acids do not show significant variations under a glucose gavage. These results disagree with those of YOKOGOSHI *et al.* (29), who observed a decrease in the plasmatic levels of branched chain amino acids in rats, after a glucose gavage. The increased plasma concentration of Trp agrees with the increased plasma ratio Trp/large neutral amino acids described after oral administration of glucose (16).

The alterations on plasma amino acid concentrations of young rats are different when glucose is added to cafeteria diet or standard diet. The administration of glucose would be expected to reduce the need for amino acids in liver gluconeogenesis. The results shown here agree with this idea in the increases shown in the plasmatic levels of the gluconeogenic amino acids Ser, Thr and Asn+Asp, and with the inhibition of serine dehydratase (an index of gluconeogenesis from amino acids) (22) caused by glucose gavage in standard diet fed rats. The normal metabolic adaptation of liver implies reciprocal changes in the level of enzymes involved in gluconeogenesis and lipogenesis (28). In standard diet fed rats the urea levels are similar, and are even higher after glucose gavage (22); if gluconeogenesis is depressed the C-backbone of some amino acids is probably directed to lipid synthesis, although some of the surplus carbohydrate may be dissipated by adaptive thermogenic mechanisms (26). In this sense, rats given glucose have lower weights than rats given saline in both diets used (22). Some experiments suggest that juvenile rats are less susceptible than ma-

ture rats to sucrose-induced obesity (13, 15, 20).

Conversely, the effect of glucose gavage on cafeteria diet fed rats is to decrease some plasma amino acid concentrations with the exception of Lys. The gluconeogenic amino acids, which are increased in the plasma by feeding the rats a cafeteria diet, do not show an additive effect with glucose gavage, as serine dehydratase was not further inhibited in the already suppressed activity of the cafeteria diet fed pups (22). ACHESON *et al.* (1) stated that, after ingestion of carbohydrate, carbohydrate oxidation and conversion to fat was significantly less and, conversely, there was a greater glycogen storage in men fed a high-fat diet. In general terms, glucose gavage has different effects on plasma amino acid concentrations depending on diet supplied, although a predominant role of diet over gavage is shown. Finally, although changes in nitrogen metabolism have been shown for the treatments supplied here (22), there is a remarkable tendency to keep constant the plasma amino acid levels.

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Se administra oralmente una solución de glucosa o una solución salina a crías de rata alimentadas con dieta normal de laboratorio o con una dieta de cafetería. Se determinan las concentraciones de aminoácidos en plasma mediante métodos radiactivos. En ratas alimentadas con dieta estándar los niveles plasmáticos de Asn+Asp, Thr, Pro, Cit, Trp y Phe son más altos en las que reciben la solución de glucosa que en las que se administra la solución salina; también los valores plasmáticos de taurina (el día 20) y Ser (el día 30) son más ele-

vados en esta situación. Los niveles plasmáticos de Arg y taurina en ratas sondadas con glucosa son menores que los de las ratas sondadas con solución salina, cuando éstas se alimentan con la dieta de cafetería. Las concentraciones de Tyr (el día 20), Gly y Pro (el día 30), son más bajas en esta situación. La dieta consumida durante los días previos a la sonda con glucosa puede tener un marcado efecto en varios parámetros metabólicos, concretamente en el metabolismo nitrogenado. A pesar de las diferentes dietas suministradas hay una tendencia muy notable a mantener constantes los niveles plasmáticos de los aminoácidos, lo que refleja la notable capacidad de equilibrio de la aminoacidemia frente a la manipulación dietética.

Palabras clave: Sonda con glucosa, Dieta de cafetería, Ratitas jóvenes, Aminoácidos.

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