Galbo, H., J.J. Holst, N.J. Christensen: Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. J. Appl. Physiol. 38: 70–76 (1975)
Galbo, H., J.J. Holst: The influence of glucagon on hepatic glycogen mobilization in exercising rats. Pfluegers Arch. 363: 49–52 (1976)
Galbo, H., E.A. Richter, N.J. Christensen, J.J. Holst: Sympathetic control of metabolic and hormonal responses to exercise in rats. Acta Physiol. Scand. 102: 441–449 (1978)
Girardier, L., J. Seydoux, M. Berger, A. Veicsteinas: Selective pancreatic nerve section. J. Physiol., Paris 74: 731–735 (1978)
Holst, J.J., H. Galbo, E.A. Richter: Neutralization of glucagon by antiserum as a tool in glucagon physiology. J. Clin. Invest. 62: 182–190 (1978)
Issekutz, B., M. Vranic: Significance of glucagon in the control of glucose production during exercise in dogs. Amer. J. Physiol. 238 (Endocrinol. Metab. 1): E13–E20 (1980)
Porte, D. Jr., L. Girardier, J. Seydoux, Y. Kanazawa, J. Posternak: Neural regulation of insulin secretion in the dog. J. Clin. Invest. 52: 210–214 (1973)
Richter, E.A., H. Galbo, B. Sonne, J.J. Holst, N.J. Christensen: Adrenal medullary control of muscular and hepatic glycogenolysis and of pancreatic hormonal secretion in exercising rats. Acta Physiol. Scand. 108: 235–242 (1980)
Sacca, L., R. Sherwin, P. Felig: Influences of somatostatin on glucagon- and epinephrine-stimulated hepatic glucose output in the dog. Am. J. Physiol.: Endocrinol. Metab. Gastrointest. Physiol. 5: E113–E117 (1979)
Samols, E., G. Marri, V. Marks: Promotion of insulin secretion by glucagon. Lancet 2: 415–417 (1965)
Sorenson, L., R.P. Elde, V. Seybold: Effect of norepinephrine on insulin, glucagon and somatostatin secretion in isolated perifused rat islets. Diabetes 28: 899–904 (1979)
Unger, R.H., R.E. Dobbs, L. Orci: Insulin, glucagon and somatostatin in the regulation of metabolism. Ann. Rev. Physiol. 40: 307–343 (1978)
Vranic, M., R. Kawamori: Essential roles of insulin and glucagon in regulating glucose fluxes during exercise in dogs. Diabetes 28 (Suppl. 1): 45–52 (1979)
Wahren, J.: Glucose turnover during exercise in healthy man and in patients with diabetes mellitus. Diabetes 28 (Suppl. 1): 82–88 (1979)

Requests for reprints should be addressed to: Erik A. Richter, M.D., Institute of Medical Physiology B, University of Copenhagen, The Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen N (Denmark)

Horm. Metab. Res. 13 (1981) 326–330

Metabolic Effects of Short Term Food Deprivation in the Rat

A. Palou, X. Remesar, Ll. Arola, E. Herrera and M. Alemany

Fisiologia General, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain
Bioquímica, Facultat de Ciències, Universitat de Ciutat de Mallorca, Ciutat de Mallorca, Balears, Spain, and
Bioquímica, Facultat de Química (Dependències de Tarragona), Universitat de Barcelona, Tarragona, Spain

Summary

The effects of food deprivation for up to 24 hours on plasma metabolic parameters in the rat have been studied. Liver dry weight and glycogen content dropped significantly from 3 hours of food deprivation onwards. Total muscle glycogen supplied about as much glycosyl residues or precursors as did the liver. Plasma glucose, urea, lactate and total and essential amino acids decreased significantly from 3 hours of fasting onwards. Glycerol, free fatty acids, beta-hydroxybutyrate and acetoacetate showed significant increases with fasting. Alanine, serine, arginine, threonine, aspartate plus asparagine and proline showed significant decreases with fasting. Several other amino acids showed almost nc change with fasting. Lysine, leucine plus isoleucine and taurine showed biphasic changes in their concentrations with a minimum at 6 hours and a transient recovery at 12 hours of fasting. Essential amino acids decreased more than the non essential ones. With fasting there is a shift in ammonia disposal with lower urea concentrations as nitrogen is better conserved. The results seem to suggest that there is a constant release of substrates, through liver and peripheral tissue proteolysis, that is counteracted by differential utilization of amino acids during fasting.

Key-Words: *Amino Acids – Fasting – Plasma Metabolites*

Received: 20 Jan. 1980 Accepted: 4 Aug. 1980

Introduction

Amino acids are known to be utilized in postabsorptive states both as metabolic fuels and gluconeogenic substrates (*Aikawa, Matsutaka, Yamamoto, Okuda, Ishikawa, Kawano and Matsumura* 1973; *Exton* 1972; *Felig* 1973). With starvation, other fuels, such as lipids and their derivatives are also preferentially used for energetic purposes (*Herrera and Freinkel* 1968; *Owen, Felig, Morgan, Wahren and Cahill* 1969). In the fasted state there is an increased flux of metabolites from peripheral tissues towards the liver (*Blackshear, Holloway and Alberti* 1974) which increases the rate of uptake of these materials (*Nallathambi, Goorin and Adibi* 1972). During food deprivation there is a net flux of amino acids and other metabolites from peripheral tissues towards the splanchnic bed (*Marliss, Aoki, Pozefsky, Most and Cahill* 1971; *Aikawa* et al. 1973; *Felig* 1973) which enter into the *Cori* and glucose-alanine cycles (*Felig* 1973; *Sudgen, Sharples and Randle* 1976).

With food deprivation, mammals develop an efficient mechanism for nitrogen conservation, particularly for essential amino acids. The metabolic changes during fasting, including the mechanisms for amino acids utilization, and the

0018–5043/81 0632–0326 $ 03.00 © 1981 Georg Thieme Verlag, Stuttgart · New York

centration, but did not significantly decrease hepatic glycogen depletion during exercise.

Effect of Combined Adrenodemedullation and Chemical Sympathectomy

In adrenodemedullated and chemically sympathectomized rats, the plasma concentration of insulin was consistently higher and the plasma concentration of glucagon lower than in control rats. However, compared to controls, hepatic glycogen depletion during exercise was not significantly lower in adrenodemedullated and chemically sympathectomized rats. In these rats, the blood glucose concentration did not change during exercise whether the rats were treated with glucagon antibodies or not.

Discussion

The present study has shown that in the rat, administration of antigen-stripped glucagon antibodies of high affinity decreases the exercise-induced hepatic glycogen depletion and the plasma insulin concentration after exercise (Table 1). Previous studies of hepatic glycogen depletion during exercise using glucagon antiserum have given varying results (*Galbo and Holst* 1976; *Galbo* et al. 1978). However, the antibodies used in those studies were not antigen-stripped and only reduced the concentration of free glucagon to about 35 % of control values, whereas the antigen-stripped glucagon antibodies used in the present study may reduce the plasma concentration to undetectable values (*Holst, Galbo and Richter* 1978). The more pronounced lowering of the free glucagon concentration possibly explains why we, in the present study, could demonstrate a marked inhibition of hepatic glycogen depletion when glucagon antibodies were administered. Furthermore, in the present study, the application of a liver biopsy technique made it possible to determine the exercise-induced hepatic glycogen depletion in individual rats. Accordingly, we were probably able to measure hepatic glycogen depletion more accurately than in our previous studies. The fact that in the present study hepatic glycogen depletion was decreased by glucagon antibodies more markedly in rats with an intact sympatho-adrenal system than in adrenodemedullated and sympathectomized rats (Table 1) points to a concentration-dependent effect of glucagon on hepatic glycogen breakdown, since the concentration of plasma glucagon in the latter was lower than in the former (Table 1). Others have suggested that the effect of glucagon on hepatic glucose output during exercise is permissive: In pancreatectomized, insulin-infused dogs the hepatic glucose production increased in the absence of an increase in the plasma (gastrointestinal) glucagon concentration (*Vranic and Kawamori* 1979), and the increase was only diminished when the plasma glucagon concentration was decreased below the resting concentration by infusion of somatostatin (*Issekutz and Vranic* 1980). Hepatic glycogen depletion has recently been shown to be reduced during exercise in adrenodemedullated (*Richter* et al. 1980) and in adrenodemedullated and chemically sympathectomized rats (*Galbo* et al. 1978) compared to controls. In the present study, combined adrenodemedullation and chemical sympathectomy did not significantly reduce the exercise-induced hepatic glycogen depletion, although such a trend was observed (Table 1).

The difference in the plasma insulin concentration between glucagon-antibody treated and control rats was not due to a difference in the plasma glucose concentration (Table 1), neither could it be ascribed to altered sympatho-adrenal activity in antibody-treated rats compared to controls, since glucagon antibodies decreased the insulin concentration, also, in adrenodemedullated and sympathectomized rats (Table 1). Thus, glucagon seems to stimulate insulin secretion in the exercising rat. Whether this stimulation of insulin secretion is due to a direct action of glucagon on the β-cell or is indirectly effectuated by glucagon, e.g., via glucagon-induced changes in the concentrations of metabolites influencing the β-cell, cannot be concluded from the present study.

A stimulating role of glucagon on insulin secretion at rest has previously been suggested (*Epand and Douglas* 1973; *Samols, Marri and Marks* 1965). Furthermore, in three dogs the concentration of glucagon and of insulin in plasma were higher and the glucose concentration lower during exercise with intact splanchnic nerves than during exercise after sectioning of the splanchnic nerves (*Girardier, Seydoux, Berger and Viecsteinas* 1978). It seems likely, that the lower plasma insulin concentration during exercise after cutting the splanchnic nerves was due to reduced stimulation of insulin secretion by glucagon, and not due to reduced splanchnic nerve enhancement of insulin secretion. This is so, since during exercise – a state where the overall sympathetic drive is increased – activity in the sympathetic fibers of the splanchnic nerves [which inhibit insulin secretion (*Porte* et al. 1973)] probably predominates relative to activity in the parasympathetic fibers of the splanchnic nerves [which stimulate insulin secretion (*Porte, Girardier, Seydoux, Kanazawa and Posternak* 1973)]. Also, *in vitro* experiments have suggested a paracrine-stimulating effect of glucagon on insulin secretion (*Sorensen, Elde and Seybold* 1979).

Combined adrenodemedullation and sympathectomy increased and decreased the plasma concentration of insulin and of glucagon, respectively, after exercise (Table 1). These findings were not explainable by a difference in the plasma glucose concentrations between rats with and without an intact sympatho-adrenal system (Table 1), but may be explained by the lack of a suppressive and stimulatory effect of catecholamines on insulin and glucagon secretion, respectively (*Richter* et al. 1980; *Unger, Dobbs and Orci* 1978).

In conclusion, the present findings indicate that in exercising rats, pancreatic glucagon enhances hepatic glycogenolysis. Furthermore, the findings suggest that glucagon and the sympatho-adrenal system increase and decrease, respectively, insulin secretion during exercise.

References

Epand, R.M., R.J. Douglas: The effect of glucagon antibodies on plasma glucose and insulin levels. Biochim. Biophys. Acta 320: 741–744 (1973)

Felig, P., J. Wahren: Role of insulin and glucagon in the regulation of hepatic glucose production during exercise. Diabetes 28 (Suppl. 1): 71–75 (1979)

sparing effect that glucose induces on amino acids oxidation, have been extensively studied (*Thompson, Schurr, Henderson and Elvehjem* 1950; *Wu* 1954; *Adibi* 1968; *Owen* et al. 1969; *Felig, Owen, Wahren and Cahill* 1969; *Cahill* 1970; *Schimmel and Knobil* 1970; *Christophe, Winand, Kutzner and Hebbelink* 1971; *Aoki, Muller, Brennan and Cahill* 1975) especially in human subjects during therapeutic long-term fasting (*Adibi* 1968; *Felig* et al. 1969; *Adibi* 1971; *Boomgardt and MacDonald* 1969; *Felig, Kim, Lynch and Hendler* 1972; *O'Connell, Morgan, Aoki, Bell and More* 1974).

In starvation, there is a rapid enhancement of gluconeogenesis (*Schimmel and Knobil* 1970), which induces alterations in the levels of circulating amino acids. Most of the studies published on this phenomenon deal with long-term fasting. The changes seen during short-term fasting are difficult to isolate from other factors known to affect the plasma concentration of these metabolites, particularly the feeding and internal circadian rhythms (*Funabiki, Ide, Ebe, Taguchi and Yasuda* 1973; *Tagliamonte, Gessa, Biggio, Vargiu and Gessa* 1974). The purpose of the present work is to determine the effect of short periods of fasting upon plasma metabolites in the rat, especially amino acid concentrations.

Materials and Methods

Female virgin Wistar rats weighing initially 174 ± 5 g were used. The animals were housed in collective cages with wire-mesh bottoms (in order to prevent coprophagia). The cages were kept in a temperature (23 ± 1 °C) and light controlled (12 hours on/12 hours off) environment. The animals were randomly divided into groups and their food (rat chow pellets) was withdrawn at 0, 3, 6, 12, and 24 hours before the onset of the light cycle. During this period the rats had free access to tap water. At the end of the fasting period the rats were killed by decapitation, taking special care not to stress the animals by handling (*Arola, Palou, Remesar, Herrera and Alemany* 1980). Blood was collected from a neck wound into dry heparinized plastic beakers.

Immediately after decapitation, pieces of liver and hind leg striated muscle were dissected and frozen in liquid nitrogen, weighed, and used for glycogen purification (*Good, Kramer and Somogyi* 1933), and glucose content determination (*Hugget and Nixon* 1957) after acid hydrolysis. The dry weight and the water content of the tissue samples were determined by differential weighing on aluminium foil before and after dessication for 36 hours in an oven at 105 ± 2 °C. Blood samples were also collected directly into heparinized tubes for hematocrit determination. Aliquots of plasma were deproteinized with perchloric acid for the enzymatic determination of acetoacetate (*Mellanby and Williamson* 1974) beta-hydroxybutyrate (*Williamson and Mellanby* 1974) glycerol (*Eggstein and Kühlman* 1974) lactate (*Hohorst* 1965) and glucose (*Hugget and Nixon* 1957). Plasma free fatty acids were determined by the colorimetric method of *Falholt, Lund and Falholt* (1973); urea was determined by the method of *Fawcett and Scott* (1960), and total plasma proteins were estimated with the *Folin* phenol reagent (*Lowry, Rosebrough, Farr and Randall* 1951; *Wang and Smith* 1975), using defatted bovine serum albumin as standard.

Small aliquots of plasma were deproteinized with cold acetone in capillary tubes (*Arola, Herrera and Alemany* 1977), and used for the radiochemical determination of individual amino acids, as previously described (*Arola, Palou, Herrera and Alemany* 1976). Most amino acid were estimated individually, except for methionine plus ornithine, glutamate plus glutamine, aspartate plus asparagine and cysteine plus cystine, due to methodological considerations (*Palou, Arola and Alemany* 1977).

All enzymes, substrates and nucleotides were purchased from Sigma Chemical Co. (St. Louis, Mo, USA), except for beta-hydroxybutyrate dehydrogenase (Boehringer Mannheim, GFR). Tests for significant differences between means were done with the Student's t test.

Results

In Table 1 the changes in body weight experienced by rats subjected to food deprivation can be seen. Fasting produced a rapid and substantial decrease in liver weight, significant from 3 hours onwards. This effect seems to be more related to a loss of dry matter than to a loss of water. There were no significant changes in the 24 hour period studied in plasma protein concentration or in striated muscle water content. The hematocrit value only showed a significant transient increase at 12 hours of food deprivation.

Liver glycogen concentration (Table 2) had already decreased considerably after 3 hours of fasting – about 50% that of controls – dropping later to about 15% that of controls at 24 hours. Muscle glycogen content was considerably smaller and there were no significant differences versus controls during the 24 hour period studied. However, there was a clear trend towards lower concentrations with time, and the mean concentration at 24 hours, was about a half of that in the fed state. The estimated glycogen content of both liver and muscle are shown in Table 2 computed from the mean body muscle mass – calculated according to *Arola, Herrera and Alemany* (1979). It has been assumed that no important changes in muscle mass (other than those proportional to changes in body weight) occur during fasting, as indicated by constant muscle water

Table 1 Body weight, liver and muscle parameters, plasma proteins and hematocrit value of rats subjected to short term food deprivation

	Hours of food deprivation				
	0	3	6	12	24
Body weight (g)	174.6 ± 5.0	171.6 ± 4.8	174.8 ± 5.2	169.8 ± 3.0	167.8 ± 4.8
Body weight loss (%)	0.0	3.5 ± 0.2*	5.6 ± 0.5*	5.7 ± 0.3*	8.0 ± 1.0*
Liver wet weight (in % of body weight)	5.26 ± 0.21	4.30 ± 0.12*	3.98 ± 0.08*	3.63 ± 0.15*	3.43 ± 0.10*
Liver dry weight (in % of body weight)	1.46 ± 0.08	1.19 ± 0.02*	1.10 ± 0.03*	1.02 ± 0.04*	0.96 ± 0.06*
Liver water content (%)	72.1 ± 0.4	71.2 ± 0.3	70.5 ± 0.4*	70.2 ± 0.4*	69.4 ± 0.2*
Striated muscle water content (%)	76.0 ± 0.6	76.0 ± 0.6	76.3 ± 0.3	75.8 ± 0.6	76.2 ± 0.3
Total plasma proteins (g/l)	69.0 ± 1.8	75.2 ± 0.9	70.4 ± 1.2	72.4 ± 1.0	71.9 ± 1.1
Hematocrit (%)	44.7 ± 1.1	43.6 ± 1.5	47.2 ± 0.9	49.1 ± 1.2*	46.6 ± 1.4

All data are the mean ± s.e.m. of 5–6 different animals per group
Significance of the differences versus fed controls (0 hours): * = $p < 0.05$

Table 2 Glycogen stores in liver and striated muscle in rats subjected to short term food deprivation

	Hours of food deprivation				
	0	3	6	12	24
Glycogen content in the liver (μmoles of glycosyl residues/g tissue)	238 ± 25	107 ± 22*	83 ± 10*	52 ± 7*	36 ± 6*
Mean glycogen content in whole liver (μmoles of glycosyl residues)#	2128	746	512	284	173
Cumulative loss (μmoles of glycosyl residues)		1382	1616	1844	1955
Glycogen content in striated muscle (μmoles of glycosyl residues/g tissue)	46 ± 9	28 ± 6	23 ± 7	27 ± 7	22 ± 2
Mean glycogen content in whole striated muscle (μmoles of glycosyl residues)#	3350	2141	1813	1826	1191
Cumulative loss (μmoles of glycosyl residues)		1209	1537	1524	2159

corrected to the initial weight of controls
All data are the mean ± s.e.m. of 5–6 different animals per group
Significance of the differences versus fed controls: * = $p < 0.05$

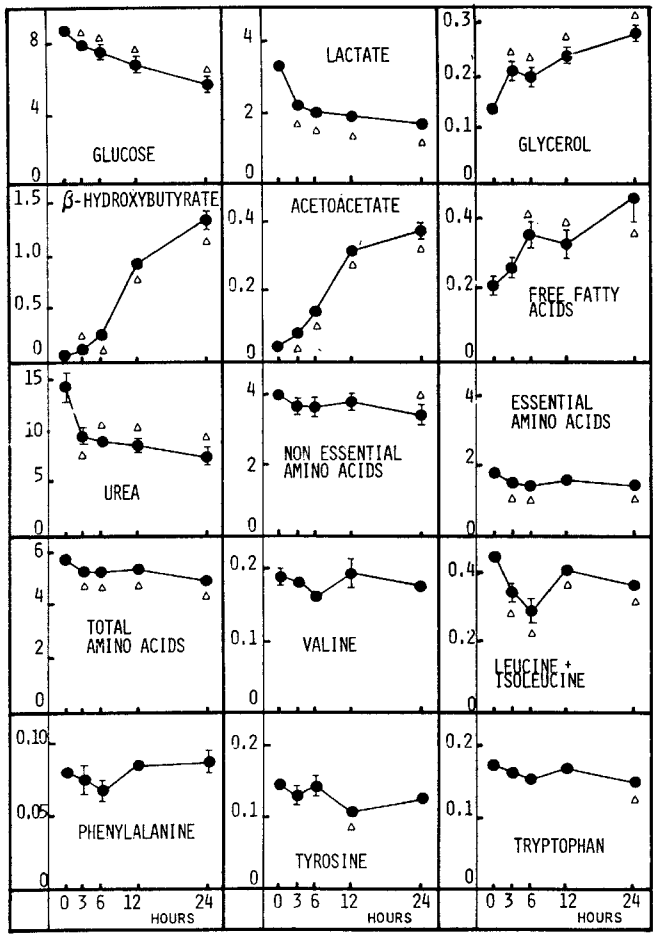

Fig. 1 Effects of short term food deprivation on the rat plasma levels of glucose, lactate, glycerol, ketone bodies, free fatty acids, urea, composite non-essential, essential and total amino acids, branched chain and aromatic amino acids.
All values are expressed in mmoles/l (except individual amino acids, expressed in μmoles/l), and are the mean ± s.e.m. of 5–6 different animals.
Significance of the differences versus fed controls: △ = $p < 0.05$

content. The cumulative loss of liver glycogen during fasting is roughly equal to that of muscle glycogen.

Plasma urea, lactate and glucose concentrations (Figure 1) showed significant decreases within 3 hours, followed by a steady decline in concentration up to 24 hours.

There was a rapid initial increase in glycerol concentration during the first 3 hours, followed by a slower increase up to 24 hours. Plasma free fatty acids followed a similar pattern. There was a continuous increase in plasma acetoacetate and beta-hydroxybutyrate with fasting; the changes being significant from 3 hours onwards.

The changes in individual and composite amino acid concentrations with fasting can be seen in Figure 1 and 2. The drop in concentration of both total and essential amino acids with fasting was significant within 3 hours, with a transient recovery at 12 hours followed by a further small but significant drop at 24 hours. The non-essential amino acids did not show any significant change until 24 hours at which time they were significantly decreased.

Discussion

The experimental scheme used actually corresponds to a post-prandrial situation, as the mean food transit time in the rat digestive system is similar to the shortest experimental food deprivation time used. However, important changes in plasma and liver composition of the rat are observed with so short a food deprivation time.

Liver and body size changes observed during food deprivation – that more directly affect changes in liver dry matter (*Kaplan and Chaikoff* 1936) – together with the absence of detectable hemoconcentration, suggest that liver protein content decreases substantially during fasting in agreement with the findings of *Herrera, Knopp and Freinkel* (1969).

The relative maintenance of plasma glucose concentration is based on the balance between the increase in liver glycogenolysis and gluconeogenesis, as well as a decrease in glucose utilization, mainly through substitution by other substrates.

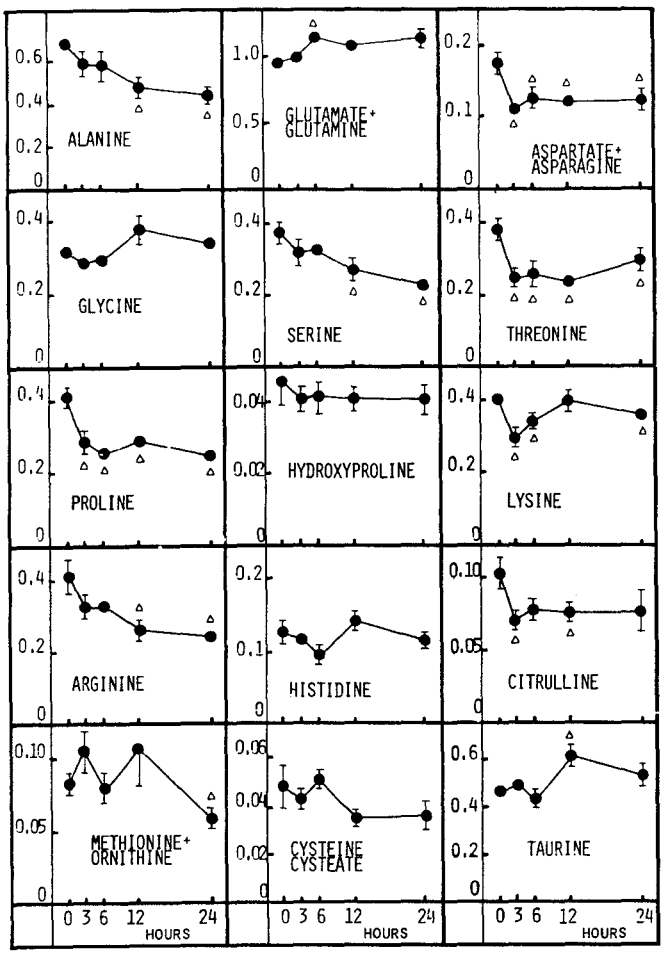

Fig. 2 Effects of short term food deprivation on the rat plasma levels of gluconeogenic amino acids, amino acids and basic and sulphur amino acids.
All values are the mean ± s.e.m. of 5–6 different animals; the results are expressed in μmoles/l.
The symbols used are the same as in Figure 1

The provision of glucose, at least during the very first stages of food deprivation, depends mainly on liver glycogenolysis. However, when the total liver and muscle glycogen content are calculated, it is apparent that by 24 hours of food deprivation, both glycogen stores have contributed roughly in the same amount of glucose or gluconeogenic precursors.

Gluconeogenesis from peripheral tissue released amino acids, lactate and glycerol is another important factor in maintaining an adequate supply of glucose during brief periods of starvation. Gluconeogenesis from lactate is enhanced from 3 hours to 12 hours of fasting in the rat (*Schimmel and Knobil* 1970), coinciding with the maximal incidence of lactate transport and uptake by the liver. In spite of the increased lactate extraction (*Kreisberg, Pennington and Boshell* 1970), plasma lactate levels are relatively constant from about 3 to 6 hours until 24 hours, in agreement with the known actuation of the *Cori* cycle.

The availability of glycerol is considerably enhanced during fasting, due to an increased adipose tissue lypolysis (*Knopp, Herrera and Freinkel* 1970) that also frees fatty acids. In the rat, fasting induces a four to five-fold increase in glucose synthesis from glycerol (*DeFreitas and Depocas* 1970; *Friedman, Goodman and Weinhouse* 1970).

The extraordinary increase in plasma ketone bodies with fasting is mainly due to increased fatty acid metabolism by the liver (*Bates, Linn and Huen* 1976) favoring the use of ketone bodies for energetic purposes, thus freeing glucose for use by the obligatory glycolytic tissues (*Cahill* 1970).

Plasma urea concentration decreased throughout the 24 hour period of fasting in agreement with *Owen* et al. (1969) and *Cahill* (1970) as a consequence of the decrease in dietary amino acid availability, together with ketone body-induced acidosis counterbalanced by an increase in ammonia production (*Herrera, Knopp and Freinkel* 1969), which reduces nitrogen availability for urea synthesis.

From the data presented it can be postulated that amino acid utilization is increased during the first hours of fasting not being completely compensated then by amino acid release from peripheral tissues (*Schimmel and Knobil* 1970). With a more prolonged fast, a variety of mechanisms for nitrogen conservation prevent waste of amino nitrogen (*Aoki* et al. 1975), explaining the partial recovery during starvation of basal concentrations of several amino acids (*Aoki* et al. 1975; *Palou, Alemany and Herrera* 1978). The decrease in plasma urea and total nitrogen excretion is consistent with the activation of nitrogen conservation mechanisms (*Cahill, Herrera, Morgan, Soeldner, Steinke, Levy, Reichard and Kipnis* 1966; *Cahill, Owen and Morgan* 1968; *Cahill* 1970). In addition, increased free ammonia concentrations favor gluconeogenesis from lactate by the liver (*Grunnet and Katz* 1978) thus sparing amino acids.

The flux of substrates from peripheral tissues towards the liver is increased in the fasted state (*Felig* 1973; *Blackshear, Holloway and Alberti* 1974), and the liver shows enhanced amino acid uptake (*Nallathambi, Goorin and Adibi* 1972); during fasting it is likely that a major fraction of amino acids released by peripheral tissues donate their alpha-amino nitrogen for synthesis of alanine, glutamine, glutamate, serine and glycine (*Aikawa* et al. 1973; *Felig* 1973). The partial recirculation of glucose carbon in the form of certain amino acids, such as alanine, through the glucose alanine cycle (*Felig* 1973) can explain the relative stability in concentration of these gluconeogenic amino acids.

The changes in amino acid concentrations in rat plasma during short term food deprivation are qualitatively different from those found during longer fasting in rats (*Henderson, Schurr and Elvejhem* 1949; *Palou, Alemany and Herrera* 1978).

The general metabolism of the rat subjected to short term food deprivation, as observed by its plasma composition, shows important changes and adaptations to the lack of food, that are detectable from 3 hours onwards. These adaptations imply the exhaustion of glycogen stores, the progressive use of lipid stores and the beginning of the metabolism of amino acids from peripheral tissues.

References

Adibi, S.A.: Influence of dietary deprivations on plasma concentrations of free amino acids of man. J. Appl. Physiol. 25: 52–57 (1968)

Adibi, S.A.: Interrelationships between levels of amino acids in plasma and tissues during starvation. Am. J. Physiol. 221: 829–838 (1971)

Aikawa, T., H. Matsutaka, H. Yamamoto, T. Okuda, E. Ishikawa, T. Kawano, E. Matsumura: Gluconeogenesis and amino acid metabolism. II. Interorganal relations and roles of glutamine and alanine in the amino acid metabolism of fasted rats. J. Biochem. (Tokyo) 74: 1003–1017 (1973)

Aoki, T.T., W.A. Muller, M.F. Brennan, G.F. Cahill: Metabolic effects of glucose in brief and prolonged fasted man. Am. J. Clin. Nutr. 28: 507–511 (1975)

Arola, Ll., A. Palou, E. Herrera, M. Alemany: Determination of plasma amino acids in small samples with the use of Dansyl-chloride. Biochimie 58: 1221–1226 (1976)

Arola, Ll., E. Herrera, M. Alemany: A new method for deproteinization of small samples of blood plasma for amino acids determination. Anal. Biochem. 82: 236–239 (1977)

Arola, Ll., E. Herrera, M. Alemany: A method for the estimation of striated muscle mass in small laboratory animals. Rev. Esp. Fisiol. 35: 215–218 (1979)

Arola, Ll., A. Palou, X. Remesar, E. Herrera, M. Alemany: Effect of stress and sampling site on metabolite concentration in rat plasma. Arch. Int. Physiol. Biochim. 88: 99–105 (1980)

Bates, M.W., L.C. Linn, A.H.J. Huen: Effects of oleic acid infusion on plasma free fatty acids and blood ketone bodies in the fasting rat. Metabolism 25: 361–373 (1976)

Blackshear, P.J., P.A.H. Holloway, K.G.M.M. Alberti: The effects of starvation and insulin on the release of gluconeogenic substrates from the extra-splanchnic tissues "in vivo". FEBS Letters 48: 310–313 (1974)

Boomgaardt, J., B.E. MacDonald: Comparison of fasting plasma amino patterns in the pig, rat and chicken. Can. J. Physiol. Pharmacol. 47: 392–395 (1969)

Cahill, G.F., M.G. Herrera, A.P. Morgan, J. Soeldner, J. Steinke, P.L. Levy, G.A. Reichard, D.M. Kipnis: Hormone fuel relationships during fasting. J. Clin. Invest. 45: 1751–1769 (1966)

Cahill, G.F., O.E. Owen, A.P. Morgan: The consumption of fuels during prolonged starvation. Adv. Enzyme Regulat. 6: 143–150 (1968)

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

Christophe, J., J. Winand, R. Kutzner, M. Hebbelinck: Amino acid levels in plasma, liver, muscle and kidney during and after exercise in fasted and fed rats. Am. J. Physiol. 221: 453–457 (1971)

Defreitas, A.S.W., F. Depocas: Glyceride-glycerol release and the interconversion of glucose and gylcerol in normal and fasted rats. Can. J. Physiol. Pharmacol. 48: 561–568 (1970)

Eggstein, M., E. Kühlman: Triglyceride and glycerol determination after alkaline hydrolysis. In: Methods of Enzymatic Analysis. Ed. Bergmeyer, H.U., pp. 1825–1831, Academic Press, New York (1974)

Exton, J.H.: Gluconeogenesis. Metabolism 21: 945–990 (1972)

Falholt, K., P. Lund, W. Falholt: An easy colorimetric micromethod for routine determination of free fatty acids in plasma. Clin. Chim. Acta 46: 105–111 (1973)

Fawcett, J.K., J.E. Scott: A rapid and precise method for the determination of urea. J. Clin. Pathol. 13: 156–163 (1960)

Felig, P., O.E. Owen, J. Wahren, G.F. Cahill: Amino acid metabolism during prolonged starvation. J. Clin. Invest. 48: 584–594 (1969)

Felig, P., T.J. Kim, V. Lynch, R. Hendler: Amino acid metabolism during starvation in human pregnancy. J. Clin. Invest. 51: 1195–1202 (1972)

Felig, P.: The glucose-alanine cycle. Metabolism 21: 197–207 (1973)

Friedman, B., E.H. Goodman, S. Weinhouse: Dietary and hormonal effects on glucogenesis from pyruvate-3-C^{14}, fructose-4-C^{14} and glycerol-2-C^{14} in the rat. Endocrinology 86: 1264–1271 (1970)

Funabiki, R., S. Ide, N. Ebe, O. Taguchi, K. Yasuda: Diurnal changes in glycogen content in skeletal muscle of mealfed rats during starvation. Agr. Biol. Chem. 39: 791–793 (1973)

Good, C.A., H. Kramer, M. Somogyi: The determination of glycogen. J. Biol. Chem. 100: 485–494 (1933)

Grunnet, N., J. Katz: Effects of ammonia and norvaline on lactate metabolism by hepatocytes from starved rats. Biochem. J. 172: 595–603 (1978)

Herrera, E., N. Freinkel: Interrelationships between liver composition, plasma glucose and ketone bodies, and hepatic acetyl-CoA and citric acid during prolonged starvation in the male rat. Biochim. Biophys. Acta 170: 244–253 (1968)

Herrera, E., R.H. Knopp, N. Freinkel: Carbohydrate metabolism in pregnancy. VI. Plasma fuels, insulin, liver composition, gluconeogenesis and nitrogen metabolism during late gestation in the fed and fasted rat. J. Clin. Invest. 48: 2260–2272 (1969)

Hohorst, H.H.: L(+)-Lactate determination with lactate dehydrogenase and NAD^+. In: Methods of Enzymatic Analysis, Ed. Bergmeyer, H.U., pp. 266–270, Academic Press, New York (1965)

Hugget, A.St.G., D.A. Nixon: Use of glucose oxidase, peroxidase, and o-dianisidine in determination of blood and urinary glucose. Lancet ii: 368–370 (1957)

Kaplan, A., I.L. Chaikoff: The relation of glycogen, fat and protein to water storage in the liver. J. Biol. Chem. 116: 663–683 (1936)

Knopp, R.H., E. Herrera, N. Freinkel: Carbohydrate metabolism in pregnancy. VIII. Metabolism of adipose tissue isolated from fed and fasted rats during late gestation. J. Clin. Invest. 49: 1438–1446 (1970)

Kreisberg, R.A., L.F. Pennington, B.R. Boshell: Lactate turnover and gluconeogenesis in normal and obese humans. Effects of starvation. Diabetes 19: 53–63 (1970)

Lowry, O.H., N.J. Rosebrough, A.L. Farr, R.J. Randall: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275 (1951)

Marliss, E.B., T.T. Aoki, T. Pozefsky, A.S. Most, G.F. Cahill: Muscle and splanchnic glutamine and glutamate metabolism in post-absorptive and starved man. J. Clin. Invest. 50: 814–818 (1971)

Mellanby, J., D.H. Williamson: Acetoacetic acid. In: Methods of Enzymatic Analysis. Ed. Bergmeyer, H.U., pp. 1840–1843, Academic Press, New York (1974)

Nallathambi, S.A., A.M. Goorin, S.A. Adibi: Hepatic and skeletal muscle transport of cycloleucine during starvation. Am. J. Physiol. 223: 13–19 (1972)

O'Conell, R.C., A.P. Morgan, T.T. Aoki, M.R. Ball, F.D. More: Nitrogen conservation in starvation: graded responses to intravenous glucose. J. Clin. Endocrinol. Metab. 39: 791–793 (1974)

Owen, O.E., P. Felig, A.P. Morgan, J. Wahren, G.F. Cahill: Liver and kidney metabolism during prolonged starvation. J. Clin. Invest. 48: 574–583 (1969)

Palou, A., Ll. Arola, M. Alemany: Plasma amino acid concentration in pregnant rats and in 21 days old foetuses. Biochem. J. 166: 49–55 (1977)

Palou, A., M. Alemany, E. Herrera: Effect of sulfonyl-urea treatment and fasting on plasma amino acids in the rat. Horm. Metab. Res. 10: 482–489 (1978)

Schimmel, R.J., E. Knobil: Insulin, free fatty acids and stimulation of hepatic gluconeogenesis during fasting. Am. J. Physiol. 218: 1540–1547 (1970)

Sudgen, M.C., S.C. Sharples, P.J. Randle: Carcass glycogen as a potential source of glucose during short-term starvation. Biochem. J. 160: 817–819 (1976)

Tagliamonte, A., R. Gessa, G. Biggio, L. Vargiu, G.L. Gessa: Daily changes of free serum triptophan in humans. Life Sci. 14: 349–354 (1974)

Thompson, H.T., P.E. Schurr, L.M. Henderson, C.A. Elvehjem: The influence of fasting and nitrogen deprivation on the concentrations of free amino acids in rats tissues. J. Biol. Chem. 182: 47–53 (1950)

Wang, C.S., R.L. Smith: Lowry determination of protein in the presence of Triton X-100. Anal. Biochem. 63: 414–417 (1975)

Williamson, D.H., J. Mellanby: Beta-hydroxybutyric acid. In: Methods of Enzymatic Analysis. Ed. Bergmeyer, H.U., pp. 1836–1839, Academic Press, New York (1974)

Wu, C.: Metabolism of free amino acids in fasted and zein-fed rats. J. Biol. Chem. 207: 775–786 (1954)

Requests for reprints should be addressed to: Prof. Dr. Marià Alemany, Bioquímica, Facultat de Ciències, Universitat de Ciutat de Mallorca, Ctra. Valldemossa km 7,5, Ciutat de Mallorca, Balears (Spain)