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Metabolic Effects of Short Term Food Deprivation in the Rat

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Introducción
Amino acids are known to be utilized in postabsorptive states both as metabolic fuels and glucoseogenic 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 splanchic bed (Marliss, Aoki, Pofesky, 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
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 adrenomedullated 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 $\beta$-cell or is indirectly effectuated by glucagon, e.g., via glucagon-induced changes in the concentrations of metabolites influencing the $\beta$-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 Viecestin 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, Eldle and Seybold 1979).

Combined adrenomedullation 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-renal 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


sparking effect that glucose induces on amino acids oxidation, have been extensively studied (Thompson, Schurr, Henderson and Ebelfjern 1950; Wu 1954; Adibi 1968; Owen et al. 1969; Felig, Owen, Wahren and Cahill 1969; Cahill 1970; Schimmel and Knoblo 1970; Christofo, Wi- nand, Kutzner and Hefbelnik 1971; Aoki, Muller, Brennan and Cahill 1975) especially in human subjects during therapeu- tic long-term fasting (Adibi 1968; Felig et al. 1969; Adibi 1971; Boograv 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 gluconeogene- sis (Schimmel and Knoblo 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 (Fumabiki, Ide, Ebe, Taguchi and Yasuda 1972; Tagliamone, Gessa, Biggio, Vargiu and Gessa 1974). The purpose of the present work is to deter- mine 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 Ale- many 1980). Blood was collected from a neck wound into dry he- parinized 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 (Huggett 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 de- proteinized with perchloric acid for the enzymatic determination of acetoacetate (Mellany and Williamson 1974) beta-hydroxybuty- rate (Williamson and Mellany 1974) glycerol (Eggerstein and Kuhl- man 1974) lactate (Hohorst 1965) and glucose (Huggett and Nixon 1957). Plasma free fatty acids were determined by the colorimetric method of Falboli, Lund and Falboli (1973); urea was determined by the method of Fawcett and Scott (1960), and total plasma pro- teins were estimated with the Folin phenol reagent (Lowry, Rose- brough, Farr and Randall 1951; Wang and Smith 1975), using de- fatted 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 prev- ously described (Arola, Palou, Herrera and Alemany 1976). Most amino acid were estimated individually, except for methionine, 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-hydroxybuty- rate dehydrogenase (Boehringer Mannheim, GFR). Tests for signi- ficant 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 stri- nated 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 de- creased considerably after 3 hours of fasting — about 50% that of controls — dropping later to about 15% that of controls at 24 hours. Muscle glycogen concentration was consid- erably 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 that of 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>
<thead>
<tr>
<th>Table 1</th>
<th>Body weight, liver and muscle parameters, plasma proteins and hematocrit value of rats subjected to short term food deprivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>174.8 ± 5.0</td>
</tr>
<tr>
<td>Body weight loss (%)</td>
<td>0.0</td>
</tr>
<tr>
<td>Liver wet weight</td>
<td>5.26 ± 0.21</td>
</tr>
<tr>
<td>Liver dry weight in (% of body weight)</td>
<td>1.46 ± 0.08</td>
</tr>
<tr>
<td>Liver water content (%)</td>
<td>72.1 ± 0.4</td>
</tr>
<tr>
<td>Striated muscle water content (%)</td>
<td>76.0 ± 0.6</td>
</tr>
<tr>
<td>Total plasma proteins (g/l)</td>
<td>69.0 ± 1.8</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44.7 ± 1.1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
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<th>3</th>
<th>6</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen content in the liver (μmoles of glycogen residues/g tissue)</td>
<td>238 ± 25</td>
<td>107 ± 22*</td>
<td>83 ± 10*</td>
<td>52 ± 7*</td>
<td>36 ± 6*</td>
</tr>
<tr>
<td>Mean glycogen content in whole liver (μmoles of glycogen residues)</td>
<td>2128</td>
<td>746</td>
<td>512</td>
<td>284</td>
<td>173</td>
</tr>
<tr>
<td>Cumulative loss (μmoles of glycogen residues)</td>
<td>1382</td>
<td>1816</td>
<td>1844</td>
<td>1955</td>
<td></td>
</tr>
<tr>
<td>Glycogen content in striated muscle (μmoles of glycogen residues/g tissue)</td>
<td>46 ± 9</td>
<td>28 ± 6</td>
<td>23 ± 7</td>
<td>27 ± 7</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>Mean glycogen content in whole striated muscle (μmoles of glycogen residues)</td>
<td>3350</td>
<td>2141</td>
<td>1813</td>
<td>1826</td>
<td>1191</td>
</tr>
<tr>
<td>Cumulative loss (μmoles of glycogen residues)</td>
<td>1209</td>
<td>1537</td>
<td>1524</td>
<td>2159</td>
<td></td>
</tr>
</tbody>
</table>

# 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, glyceral, 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 roughy 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 drug in concentration of both total and essential non-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-prandial 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 hemocoencentration, 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 glycolysis and gluconeogenesis, as well as a decrease in glucose utilization, mainly through substitution by other substrates.
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 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 Boschell 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 lipolysis (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 Depocps 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, Alemamy 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 (Felg 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; Felg 1973). The partial recirculation of glucose carbon in the form of certain amino acids, such as alanine, through the glucose alanine cycle (Felg 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 Elvehem 1949; Palou, Alemamy 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


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