Molecular Physiology, 2 (1982) 193–201
Elsevier Biomedical Press

EFFECT OF FASTING ON THE CIRCULATING GLUCOSE AND INSULIN LEVELS AFTER GLUCOSE, ARGININE, PYRUVATE AND PALMITATE ADMINISTRATION IN THE RAT

J. CODINA[a] and E. HERRERA[b,*]

[a] *Cátedra de Fisiología General, Facultad de Biología, Universidad de Barcelona and* [b] *Departamento de Investigación, Centro 'Ramón y Cajal', Madrid, Spain.*

(Received 5 July 1981, accepted 8 October 1981)

Key words: Fasting; Glucose; Insulinotropic agents; In vivo insulin release

Summary

The effect of glucose, arginine, pyruvate and palmitate administration on levels of circulating glucose and RIA-insulin was studied in fed and 48 h-fasted rats. The rise in blood glucose level after oral glucose load was greater in fasted than in fed rats, whereas plasma insulin level increase was similar in both groups. When glucose was given intravenously, plasma RIA-insulin rose only in the fed animals. Arginine administration produced minor changes in these parameters in both fed and fasted rats. Oral pyruvate produced greater enhancement in blood glucose concentration in fasted than in fed animals while plasma insulin levels rose only in the fed rats. After palmitate load, blood glucose levels increased only in the fed animals in which there was also an increase in plasma insulin levels following intravenous administration of fatty acid. These results suggest that none of the metabolites used except glucose has a physiological role in insulin secretion in the fed or fasted animals; in the latter group sensitivity to glucose stimulus was greatly reduced while the release of insulinotropic gastrointestinal factors after administration of oral glucose appeared less affected. The changes in blood glucose levels observed after addition of pyruvate or palmitate are discussed in terms of the role of pyruvate as a gluconeogenetic substrate and of the effect of palmitate on glucose metabolism.

Introduction

It is well known that the reduced tolerance to carbohydrate in the fasting state results mainly from diminished insulin release (Cahill et al., 1966; Malaisse et al., 1967; Bosboom et al., 1973). The mechanism through which this impaired islet-cell response is exerted

* To whom correspondence should be addressed: Departamento de Investigación, Centro 'Ramoń y Cajal', Ctra. de Colmenar km 9, Madrid 34, Spain.

0166-6851/82/0000–0000/$02.75

during fasting is not yet clear. Some authors have concluded that the effect may result from a lack of available glucose (Grey et al., 1970; Hedeskow and Capito, 1974) while others suggest that intestinal hormones play an important role in insulin release during fasting (Turner and Young, 1973). More recently it has been proposed that a reduced capacity of the secretory mechanism rather than its sensitivity to glucose is the main factor contributing to impaired insulin release in the fasting state (Joost and Beckmann, 1980). During fasting there are great changes in the blood concentration of various metabolites including amino acids (Felig et al., 1972; Eisenstein et al., 1979), lactate and pyruvate (Williamson et al., 1967); Konijn et al., 1976) and free fatty acids (Foster, 1967; Schimmel and Knobil, 1969; Owen et al., 1973). As some of these metabolites have been reported to enhance pancreatic insulin in in vitro preparations (Edgar et al., 1969; Hellman et al., 1972; Loubatieres-Mariani et al., 1974; Goberna et al., 1974; Loubatieres-Mariani et al., 1976; Campillo et al., 1979) this study was performed to determine whether they also effect insulin secretory activity when administered intravenously or orally to fed and fasted rats. The mechanism of impaired insulin release in fasting was investigated as well as the possible importance of metabolites other than glucose in the in vivo β-cell function during food deprivation.

Material and Methods

Female Wistar rats, weighing 140–150 g were maintained at 22 ± 1°C with controlled (12 h on-off) light cycles. The animals were fed a standard purina chow diet and when deprived of food were allowed water 'ad libitum'. To determine the pancreas insulin content, animals were killed by guillotine and the pancreas was rapidly dissected for insulin extraction (Duran-García et al., 1976) and assayed by radioimmunoassay (Heding, 1972) with an insulin radioimmunoassay kit for the rat, generously supplied by Novo Industria/S. Other animals were treated with equicaloric amounts of either glucose, arginine or pyruvate, or with palmitate administered either by intravenous injection through a vein in the tail or by gastric sonda. The intravenous doses were always one-tenth of the oral doses because preliminary experiments with glucose showed that with this amount similar maximal blood glucose concentrations were obtained during oral and intravenous tests. With the exception of palmitate, all other administered compounds were dissolved in distilled water. Palmitate for oral administration was suspended in a carboxymethylcellulose solution (0.5%) supplemented with Tween-80 (0.125%). For intravenous administration, palmitate was dissolved in 8% bovine albumin, previously purified (Chen, 1957). Blood was always collected from the tip of the tail into heparinized vials. Aliquots of blood were used for plasma separation to determine the RIA-insulin by the procedure indicated above or to precipitate the proteins by the Somogyi method (1945). Glucose was evaluated in the protein-free supernatants with the glucose oxidase procedure (Hugget and Nixon, 1957). Statistical analysis of the data was performed with the Student t-test.

Results

Pancreatic insulin concentration. The amount of insulin found in ethanol pancreatic extracts was slightly but significantly lower in the 48 h-fasted rates (1.25 ± 0.06 U/pancreas) than in their fed controls (1.65 ± 0.14 U/pancreas); ($P = 0.05$).

Glucose tolerance tests. Oral responses to 2 g/kg glucose were studied in fed and 48 h-fasted rats. Blood glucose levels after the oral glucose load (Fig. 1A) increased more in fasted than in fed rats. In the latter group, blood glucose levels returned to basal values after 30 min while in the fasted animals they remained significantly elevated even after 60 min and returned to normal at 120 min. Basal plasma RIA-insulin levels were higher in the fed than in the fasted animals (Fig. 1B), but in both groups the response to the glucose load was similar, with increases in plasma insulin at 3, 7.5, 15, 22.5 and 30 min when compared with basal levels. Intravenous administration of 0.2 g/kg glucose caused an initial rapid rise in blood glucose levels similar in fed and fasted animals (Fig. 1C), and the subsequent decrease of blood glucose up to 60 min after the intravenous load was also similar in both groups. At 120 min, blood glucose levels were significantly lower, in the fasted animals (P = 0.05). The increased levels of circulating insulin after intravenous glucose administration were observed in the fed animals until 30 min (Fig. 1D) and plasma levels were significantly higher in comparison with basal values. On the contrary, in fasted rats there was no significant increment in plasma insulin levels after intravenous glucose administration at any of the times studied (Fig. 1D).

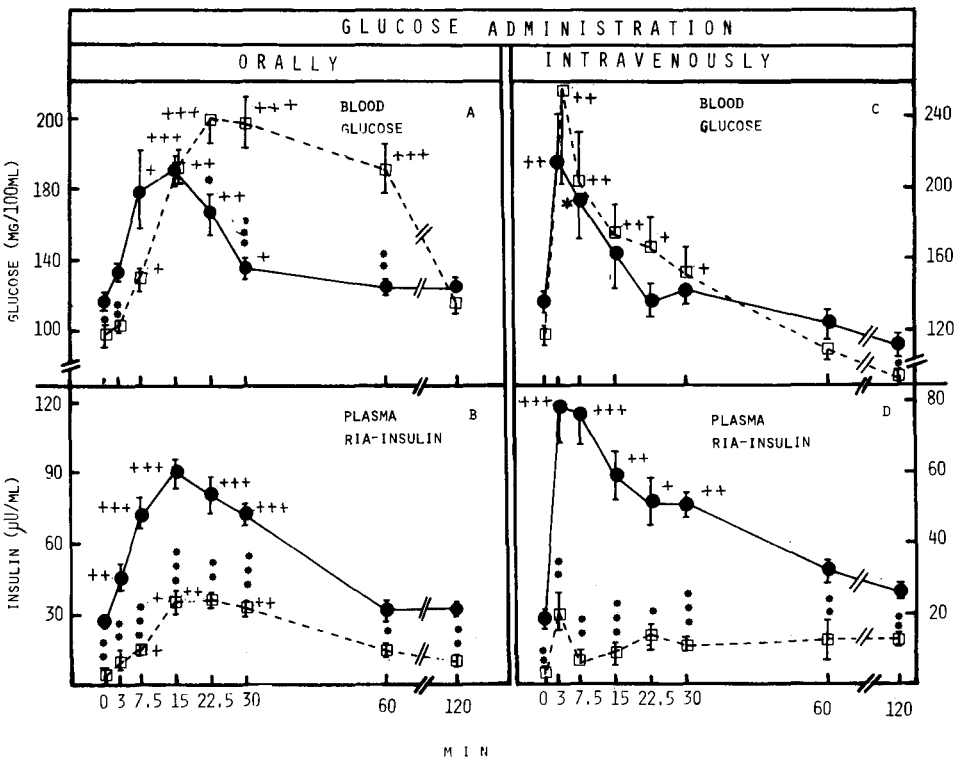

Fig. 1. Blood glucose and plasma RIA-insulin concentration in fed (●—●) and 48 h-fasted rats (□- -□) after oral (2 g/kg body weight) or intravenous (0.2 g/kg body weight) administration of glucose. Means ± S.D. of 6–8 rats/group. Statistical comparisons are shown by asterisks: Fed vs. fasted: *, P = 0.05; **, P = 0.01; ***, P = 0.001; P vs. zero min: +, P = 0.05; ++, P = 0.01; +++, P = 0.001.

Arginine administration. After either oral or intravenous administration of arginine, blood glucose and plasma insulin levels did not change significantly in the fed rats (Fig. 2). At 48 h, both blood glucose and plasma insulin levels were significantly reduced in fasted as compared with fed animals. Blood glucose concentration in fasted rats had decreased significantly at 15, 30, 60 and 120 min after intravenous administration of arginine and plasma insulin levels in the fed rats were augmented at 3 min after intravenous administration, but no other changes were observed in either plasma insulin levels or blood glucose concentrations following intravenous or oral administration of arginine (Fig. 2).

Pyruvate administration. Oral pyruvate administration produced significant increases in blood glucose concentrations at 7.5, 22.5 and 30 min in fed rats and at 15, 22.5 and 30 min in fasted animals (Fig. 3A). Plasma insulin levels (Fig. 3B) were significantly augmented in the fed animals at 3 and 15 min after pyruvate oral load while they had not changed in the fasted rats. Intravenous administration of pyruvate caused a significant reduction of blood glucose levels at 60 and 120 min in fed animals (Fig. 3C) while it

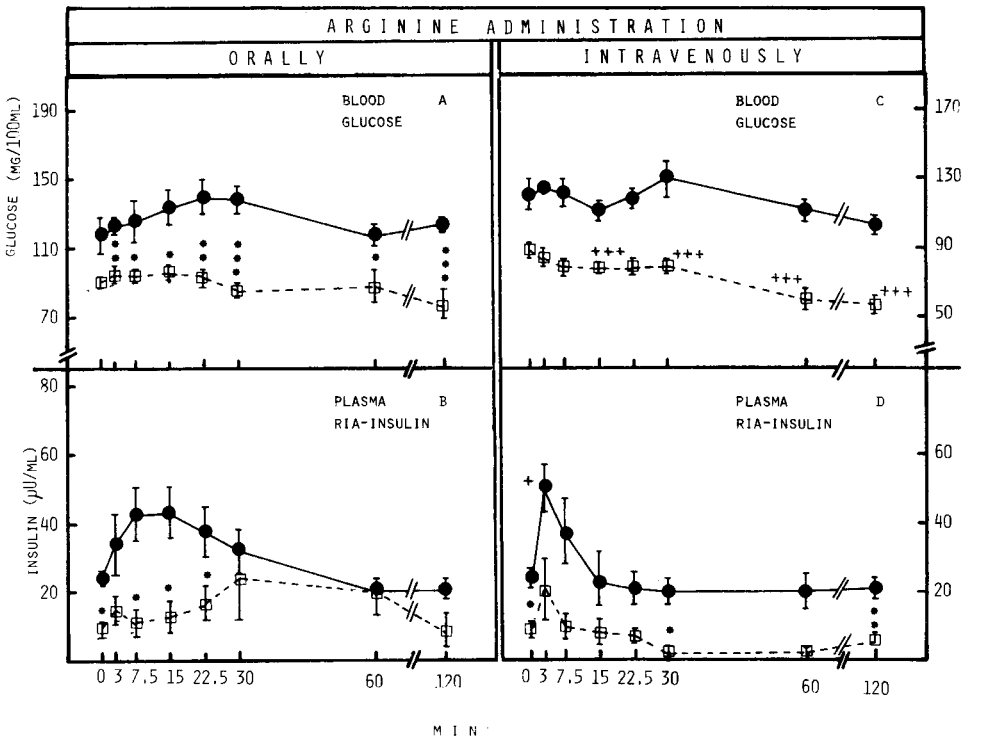

Fig. 2. Blood glucose and plasma RIA-insulin concentration in fed (●—●) and 48 h-fasted rats (□- -□) after oral (2.96 g/kg body weight) or intravenous (0.29 g/kg body weight) administration of arginine. Means ± S.D. of 6–8 rats/group. Statistical comparisons are shown by asterisks: Fed vs. fasted: *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$; P vs. zero min: +, $P = 0.05$; ++, $P = 0.01$; +++, $P = 0.001$.

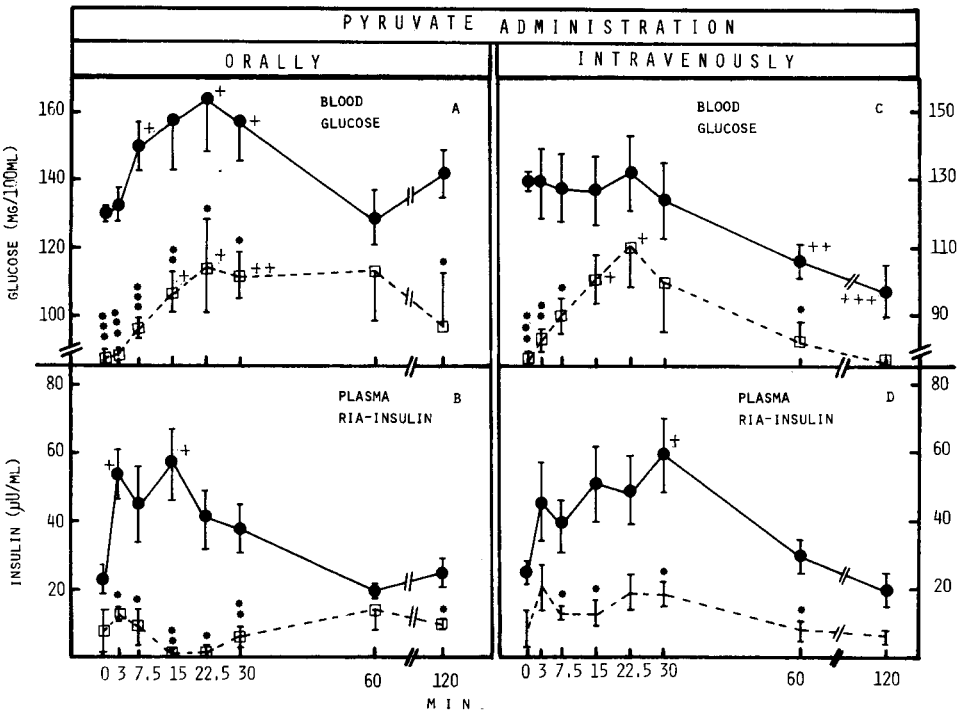

Fig. 3. Blood glucose and plasma RIA-insulin concentration in fed (●—●) and 48 h-fasted rats (□– –□) after oral (3.1 g/kg body weight) or intravenous (0.31 g/kg body weight) administration of pyruvate. Means ± S.D. of 6–8 rats/group. Statistical comparisons are shown by asterisks: Fed vs. fasted: *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$; P vs. zero min: +, $P = 0.05$; ++, $P = 0.01$; +++, $P = 0.001$.

produced a significant increase at 15 and 22.5 min in fasted rats (Fig. 3C). Plasma insulin levels were augmented 30 min after intravenous administration of pyruvate to fed but not to fasted animals.

Palmitate administration. After oral palmitate load, blood glucose levels rose significantly in fed rats at 3, 15, 22.5 and 30 min (Fig. 4A). Plasma insulin levels also rose significantly at 15 min in these animals (Fig. 4B) while neither parameter was altered in the fasted rats (Fig. 4A and B). Intravenous administration of palmitate produced a marked and rapid increment in blood glucose levels in the fed animals that lasted up to 30 min after treatment (Fig. 4C) while in fasted animals there was a significant decrease at 60 and 120 min (Fig. 4C). Except for an increase at 3 min in the fed animals, plasma insulin levels were unchanged in both fed and fasted animals.

To determine whether the medium used to solubilize the palmitate for intravenous administration (8% purified bovine albumin) was responsible for any of the observed changes, both fed and fasted rats were intravenously injected with either the medium or saline. At all times studied, the levels of blood glucose and plasma insulin did not differ in comparison with zero-time values in any group (data not shown).

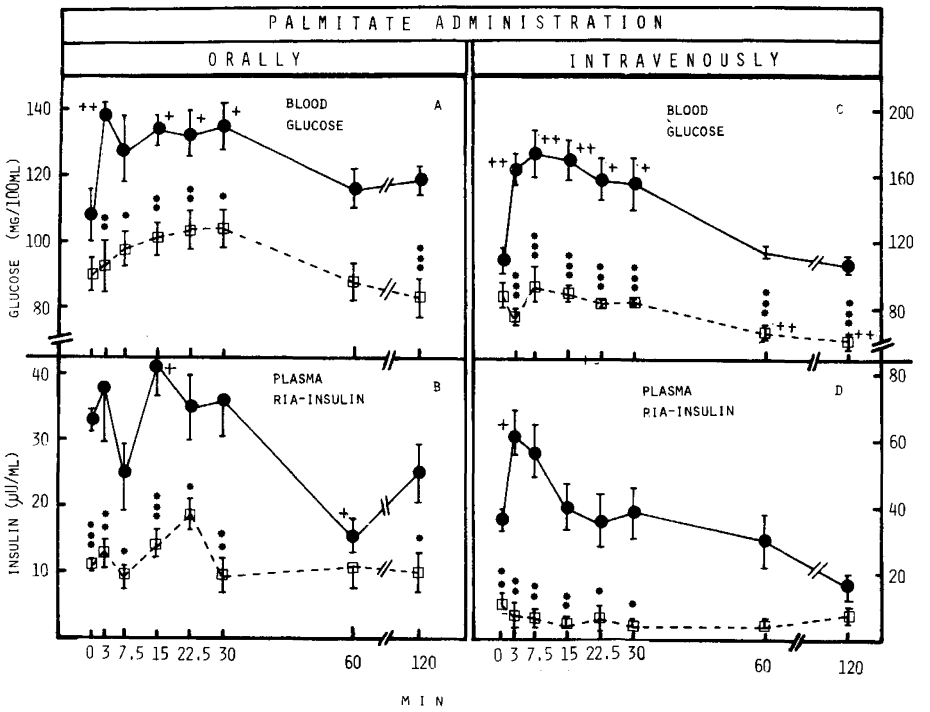

Fig. 4. Blood glucose and plasma RIA-insulin concentration in fed (●—●) and 48 h-fasted (□- - -□) rats after oral (0.83 g/kg body weight) or intravenous (2 ml saturated solution in 8% purified albumin/kg body weight) administration of palmitate. Means ± S.D. of 6–8 rats/group. Statistical comparisons are shown by asterisks: Fed. vs. fasted: *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$; P vs. zero min: +, $P = 0.05$; ++, $P = 0.01$; +++, $P = 0.001$.

Discussion

Present findings confirm earlier reports that the release of insulin following administration of oral glucose was much more a response to intravenous glucose than the effect of fasting (Turner and Young, 1973). While this observation is contrary to the results of Grey et al. (1970), the high doses of anesthetic used by these authors may have influenced the response, as already suggested (Turner and Young, 1973). The higher insulin levels observed after oral glucose administration as compared to those after intravenous administration with a similar glycemic stimulus are in agreement with previous observations (Elrick et al., 1964; McIntyre et al., 1965; Rehfeld et al., 1970; Faber et al., 1979). Although other factors beside the incretin effect may contribute to the stronger reaction to the oral glucose load (Faber et al., 1979), our findings demonstrate that they are also active in the fasting state, evoking a significant insulinotropic response, whereas the pancreas is unable to react to the hyperglycemia produced by the intravenous glucose administration. In the fasted state, there is apparently reduced sensitivity to glucose in the insulin secretory mechanism rather than a reduced capacity of the pancreas to respond to other stimuli. The insulin content of the pancreas is known to decrease during fasting

(Malaisse et al., 1967; Bosboom et al., 1973; Coddling et al., 1975; Joost and Beckmann, 1980) but it has been unanimously proposed (Malaisse et al., 1967; Buchanan et al., 1969; Bosboom et al., 1973; Coddling et al., 1975) that this diminution is independent of the insulin secretion by the pancreas because it is limited and appears more slowly during starvation. Therefore the decreased insulin content in the pancreas does not seem to be responsible for the reduced insulinotropic action of glucose and the precise mechanism of this effect remains to be established.

Acute arginine administration by gastric sonda or by intravenous injection failed to alter plasma insulin levels in either fed or fasted rats. Reported increases in plasma insulin levels after the in vivo administration of arginine in rats involved higher doses than those used here and/or infusion for prolonged periods (Kuku et al., 1978; Lippman and Kobric, 1978). Thus the insulinotropic effect of arginine does not appear to be physiologic and the slight changes in plasma levels of this amino acid during fasting (Felig et al., 1972; Eisenstein et al., 1979) are probably insufficient to effect endogenous insulin secretion.

Pyruvate administration produced significant increments in blood glucose levels in both fed and fasted rats although the effect was greater in the latter where it was observed after oral and intravenous administration. The hyperglycemic effect of pyruvate is probably due to its well known rapid conversion into glucose in the fasted state (Denton and Halesrap, 1979) and actually the in vivo gluconeogenesis from labelled pyruvate has been found greater in fasted than in fed rats (Herrera et al., 1969; Llobera and Herrera, 1980). The slight increases in plasma insulin concentration after pyruvate administration in fed animals is probably secondary to the increase in blood glucose levels, whereas the lack of change in fasted animals is consistent with their islet insensitivity to the hyperglycemic stimulus.

Palmitate administration increased blood glucose levels in fed rats and caused subsequent small rises in plasma insulin levels. These effects are similar whether given orally or intravenously and do not occur in fasted animals. Although in vivo insulinotropic effects of free fatty acids have been reported (Goberna et al., 1974; Campillo et al., 1979), present results indicate that higher endogenous levels of palmitate do not affect insulin secretion in the fed or fasted rat. The small rise in plasma insulin concentration in fed animals after palmitate administration derives from its previous hyperglycemic effect. This effect must be a consequence of either increased liver glycogenolysis and gluconeogenesis or reduced extrahepatic glucose utilization. All these changes are probably interacting simultaneously and may be augmented by greater concentrations of endogenous free fatty acids (Foster, 1967; Schimmel and Knobil, 1969). The lack of hyperglycemic response in fasting animals after palmitate administration may be due to their already low glycogen stores (Herrera and Freinkel, 1968) or to the fact that gluconeogenesis is already augmented (Herrera et al., 1969; Llobera and Herrera, 1980) and peripheral glucose consumption reduced (Freiminet et al., 1976) in such a way that there is no possibility of further change despite the fatty acid load.

Present results demonstrate that none of the metabolites used other than glucose have a physiological role in insulin secretory activity in either fed or fasted animals and do not affect the release of intestinal insulinotropic factors. In the fasted state, insulin release is insensitive even to the endogenous hyperglycemia induced by pyruvate administration and it seems that other effectors besides an increase in blood glucose must be

involved in adjustment of the β-cell function to the post-prandial state. Although these factors are not yet known, a priming effect of intestinal hormones has been proposed (Turner and Young, 1973), in which case a stimulus previous to their endogenous release would be required in the fasted animal to trigger its β-cell sensitivity to the glucose load.

Acknowledgements

The authors wish to express their gratitude to Caroline S. Delgado for her help. This study was performed in part with a grant from the Comisión Asesora de Investigación Científica y Técnica, Spain.

References

Bosboom, R.S. Zweens, J. and Bouman, P.R. (1973): Effect of feeding and fasting on the insulin secretory response to glucose and sulfonylureas in intact rats and isolated perfused rat pancreas. Diabetologia 9, 243–250.

Buchanan, K.D., Vance, J.E. and Williams, R.H. (1969): Effect of starvation on insulin and glucagon release from isolated islets of Langerhans of the rat. Metabolism 18, 155–162.

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

Campillo, J.E., Valdivia, M.M., Rodriguez, E. and Osorio, C. (1979): Effect of oleic and octanoic acids on glucose-induced insulin release in vitro. Diabetes Metabol. 5, 183–187.

Chen, R.F. (1957): Removal of fatty acids from serum albumin by charcoal treatment. J. Biol. Chem. 226, 497–509.

Coddling, J.A., Kalnius, A. and Haist, R.E. (1975): Effect of age and of fasting on the responsiveness of the insulin-secreting mechanism of the islets of Langerhans to glucose. Can. J. Physiol. Pharmacol. 53, 716–725.

Denton, R.M. and Halesrap, A.P. (1979): Regulation of pyruvate metabolism in mammalian tissues. In: Essays in Biochemistry (Campell, P.N. and Matshall, R.D., eds.), pp. 37–77. Academic Press, London.

Duran-Garc'a, S., Jarrousse, C. and Rosselin, G. (1976): Biosynthesis of proinsulin and insulin in newborn rat pancreas. Interaction of glucose, cyclic AMP, somatostatin and sulfonyloureas on the [^{3}H]leucine incorporation into immunoreactive insulin. J. Clin. Invest. 57, 230–243.

Edgar, P., Rabinowitz, D. and Merimee, T.J. (1969): Effects of amino acids on insulin release from exised rabbit pancreas. Endocrinology 84, 835–843.

Eisenstein, A.B., Strack, I., Gallosoro-Torres, H., Georgiadis, A. and Miller, O.N. (1979): Increased glucagon secretion in protein-fed rats: lack of relationship to plasma amino acids. Am. J. Physiol. 236, E20–E27.

Elrick, H., Stimmler, L., Hlad, C.J. and Arai, Y. (1964): Plasma insulin response to oral and intravenous glucose administration J. Clin. Endocrinol. 24, 1076.

Faber, O.K., Madsbad, S., Kehlet, H. and Binder, Ch. (1979): Pancreatic beta cell secretion during oral and intravenous glucose administration. Acta Med. Scand. Suppl. 624, 61–64.

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

Foster, D. (1967): Studies in the ketosis of fasting. J. Clin. Invest. 46, 1283–1296.

Ferminet, A., Poyart, C., Leclerc, L. and Gentil, M. (1976): Effect of fasting on glucose recycling in rats. FEBS Lett. 61, 294–297.

Goberna, R., Tamarit, J. Jr., Osorio, J., Fussganger, R., Tamarit, J. and Pfeiffer, E.F. (1974): Action of β-hydroxybutyrate, acetoacetate and palmitate on the insulin release in the perfused isolated rat pancreas. Horm. Metab. Res. 6, 256–260.

Grey, N.J., Goldring, S. and Kipnis, D.M. (1970): The effect of fasting, diet and actinomycin D on insulin secretion in the rat. J. Clin. Invest. 49, 881–889.

Hedeskow, C.J. and Capito, K. (1974): The effect of starvation on insulin secretion and glucose metabolism in mouse pancreatic islets. Biochem. J. 140, 423–433.

Heding, L.G. (1972): Determination of total serum insulin (IRI) in insulin treated diabetic patients. Diabetologia 8, 260–266.

Hellman, B., Sehlin, J. and Taljedal, I.B. (1972): Transport of L-leucine and D-leucine into pancreatic β-cells with reference to the mechanisms of amino acid-induced insulin release. Biochim. Biophys. Acta 266, 436.

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

Herrera, E., Knopp, R.H. and Freinkel, N. (1969): 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.

Hugget, A.St.G. and Nixon, D.A. (1957): Use of glucose oxidase, preoxidase and O-dianisidine in the determination of blood and urinary glucose. Lancet 2, 368–379.

Joost, H.G. and Beckmann, J. (1980): On the relation of glucose and insulin secretion in the fasting state. Metabolism 29, 23–27.

Konijn, A.M., Carmel, N. and Kaufmann, N.A. (1976): The redox state and the concentration of ketone bodies in tissues of rats red carbohydrate free diets. J. Nutr. 106, 1507–1514.

Kuku, S.F., Jaspan, J.B., Emmanouel, D.S., Katz, A.I. and Rubenstein, A.H. (1978): Plasma glucagon, insulin and glucose responses to intravenous arginine infusion in the rat. Horm. Metab. Res. 10, 99–100.

Lippmann, W. and Kobric, M. (1978): Effect of alrestatin on arginineinduced secretion of glucagon and insulin in the rat. Horm. Metab. Res. 10, 280–282.

Llobera, M. and Herrera, E. (1980): Effects of starvation on in vivo gluconeogenesis in hypo- and hyperthyroid rats. Endocrinology 106, 1628–1633.

Loubatieres-Mariani, M.M., Blayac, J.P., Alric, R., Valette, G. and Loubatieres, A.L. (1974): Effects du D-L lactate de sodium sur les secrétions d'insuline et de glucagon. Etude sur le pancreas isolé et perfusé de rat, C.R. Soc. Biol. 168, 1038–1044.

Loubatieres-Mariani, M.M., Loubatieres, A.L. and Blayac, J.P. (1976): Etude comparative des effects insuline-secreteurs du L (+) et du D (-) lactate de sodium. C.R. Soc. Biol. 170, 825–827.

Malaisse, W.J., Malaisse-Lagae, F. and Wright, P.H. (1967): Effect of fasting upon insulin secretion in the rat. Am. J. Physiol. 843–848.

McIntyre, N., Holdsworth, C.D. and Turner, D.S. (1965): Intestinal factors in the control of insulin secretion. J. Clin. Endocr. 25, 1317–1324.

Owen, O.E., Markus, H., Sarshik, S. and Mozzoli, M. (1973): Relationship between plasma and muscle concentrations of ketone bodies and free fatty acids in fed, starved and alloxan-diabetic states. Biochem. J. 134, 499–506.

Rehfeld, J.F., Juhl, E. and Quaade, F. (1970): Effect of jejunoileostomy on glucose and insulin metabolism in ten obese patients. Metabolism 19, 529–538.

Schimmel, R.J. and Knobil, E. (1969): Role of free fatty acids in stimulation of gluconeogenesis during fasting. Am. J. Physiol. 217, 1803–1808.

Somogyi, M. (1945): Determination of blood sugar. J. Biol. Chem. 160, 69–73.

Turner, D.S. and Young, D.A.B. (1973): The effect of fasting and selective refeeding on insulin release in the rat. Acta Endocrinol. 72, 46–53.

Williamson, D.H., Lund, P. and Krebs, H.A. (1967): The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochrondria of rat liver. Biochem. J. 103, 514–527.