University of Groningen

Reduced Insulin Secretion After Short-Term Food Deprivation in Rats Plays a Key Role in the Adaptive Interaction of Glucose and Free Fatty Acid Utilization

Strubbe, J.H.; Alingh Prins, A.J.

Published in:
Physiology & Behavior

DOI:
[10.1016/0031-9384\(86\)90203-9](https://doi.org/10.1016/0031-9384(86)90203-9)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1986

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Strubbe, J. H., & Alingh Prins, A. J. (1986). Reduced Insulin Secretion After Short-Term Food Deprivation in Rats Plays a Key Role in the Adaptive Interaction of Glucose and Free Fatty Acid Utilization. *Physiology & Behavior*, 37(3), 441-445. [https://doi.org/10.1016/0031-9384\(86\)90203-9](https://doi.org/10.1016/0031-9384(86)90203-9)

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Reduced Insulin Secretion After Short-Term Food Deprivation in Rats Plays a Key Role in the Adaptive Interaction of Glucose and Free Fatty Acid Utilization

J. H. STRUBBE AND A. J. ALINGH PRINS

Department of Animal Physiology, P.O. Box 14, 9750 AA Haren, The Netherlands

Received 17 January 1986

STRUBBE, J. H. AND A. J. ALINGH PRINS. *Reduced insulin secretion after short-term food deprivation in rats plays a key role in the adaptive interaction of glucose and free fatty acid utilization.* PHYSIOL BEHAV 37(3) 441-445, 1986.—The present study was undertaken to investigate the effects of short-term fasting periods up to 24 hr on insulin secretory responses of the B-cell to glucose and the consequences for FFA and glucose availability in the circulation. Conscious male rats provided with permanently implanted heart catheters received glucose infusions at midday, lasting for 20 min, in the nearly ad lib condition (i.e., 6 hr of non feeding during daytime) and after extending the fasting period to 12, 18 and 24 hr. Basal preinfusion insulin levels and insulin responses to glucose decreased gradually during these fasting periods. Basal blood glucose dropped only significantly after 24 hr of fasting whereas basal FFA levels increased gradually from 6 hr of fasting onwards. After prolonged fasting insulin released during glucose infusion became more effective in suppressing plasma FFA levels. While our data suggest that the sensitivity to the antilipolytic action of insulin is increased, the decreased responsiveness of the B-cell after moderate fasting periods may result in a drop of basal insulin levels. This facilitates the switch from glucose to FFA metabolism for most tissues already, when the first meals are missed. The results suggest that this physiological process is important to save the glycogen stores as long as possible as fuel for the central nervous system, and also to support basic energy requiring processes adequately.

Plasma insulin Blood glucose Free fatty acids Food deprivation Insulin secretion

IN vitro and in vivo studies of insulin secretion have shown that the insulin secretory response to glucose is markedly reduced in the fasted state [4, 21, 22, 25]. The mechanism for this phenomenon has been studied in detail and there is evidence that decreased c-AMP formation from ATP is involved [4,25]. It is also known that fasting over 24 hr is accompanied by a decrease in blood glucose and an increment in plasma free fatty acids (FFA) [13,18]. There is some evidence that in the fasted state the increased availability of FFA covers the metabolic needs of most tissues. In this state, however, the central nervous system can still utilize mainly glucose, which is produced by the liver by gluconeogenesis at a low rate [13]. During short-term fasting, glycogen in the liver may meet the metabolic needs for glucose via glycogenolysis. It is therefore important to know when during the day FFA levels begin to increase and what the responsiveness of the B-cells is over the course of fasting. Insulin has an antilipolytic action and is a potent stimulus for uptake of glucose and FFA in many tissues [6]. Changes in responsiveness of the B-cell may be reflected in changes of basal insulin levels which influence the availabil-

ity of glucose and FFA in the blood. Most studies on the fasting phenomenon, however, deal with long-term fasting. In vivo studies on short-term fasting are scarce because it is sometimes difficult to cope with the feeding rhythm and the circadian control over various endogenous rhythms of physiological processes, such as glycogenesis [15], intestinal transport [2], liver activity [23], all influencing glucose and FFA plasma levels.

The present study was therefore undertaken to investigate the effects of short-term fasting periods up to 24 hr on insulin secretory responses of the B-cell to glucose and the consequences for FFA and glucose availability in the circulation.

METHOD

Animals

Male albino Wistar rats (380–420 g) were kept solitarily in Plexiglas cages at a room temperature of 20°C. Light was on from 6 a.m. to 6 p.m. Food and water were supplied ad lib

FIG. 1. Mean food intake (g) of 6 rats over 14 days. The black area at the top indicates nighttime. Fasting times are indicated by horizontal arrows.

except during the food deprivation experiments. The diet, pelleted lab chow, contained 20% protein, 5% fat, 53.3% carbohydrate and 21.7% minerals, cellulose and water. Energy content amounted to 17.8 kJ/g. A rat could gnaw off pieces of food through vertical stainless steel bars situated in front of the food hopper. Spillage was collected in an undertray attached to the food hopper. The weight of each food hopper was sampled continuously by a programmed microprocessor such that the amount of food eaten during every minute over the day-night cycle could be ascertained. Access to food could be restricted by a horizontally sliding door situated in front of the hopper. Door opening and closing was activated by a programmed clock.

Surgery and Blood Sampling

The rats were provided with two permanent heart catheters to the right atrium. The sampling cannula was inserted into the right jugular vein and the infusion cannula into the left [17]. A small swivel joint was incorporated in the infusion tube [20]. These catheters allow blood sampling and intracardial infusion in the freely moving, undisturbed and unanesthetized rats. Cannulated rats were used for experimentation once they had become adapted to the sampling procedure, usually after about one week. Blood samples (0.5 ml) were transferred immediately to chilled (0°C) centrifuge tubes containing 10 μ l heparin solution (500 U/ml) as anticoagulant. To avoid clotting of blood during the sampling procedure 6% citrate solution was used instead of heparin which causes the activation of lipoprotein lipase. After each sample, 0.5 ml of citrated blood of a donor rat was transfused through the sample catheter.

Experiments

The experiments were performed on six unanesthetized and undisturbed rats. Intravenous glucose infusions were given to test the responsiveness of the B-cell since oral feeding would interact with different intestinal contents. The latter may release hormonal and other gut factors which may influence the results. In order to prevent differences due to circadian effects, intracardial glucose infusions were all given at the same time at the middle of the light phase by infusion of 10% (w/v) glucose in distilled water over a period of 20 min at a rate of 10 mg/min. The infusion was given after 6 hr of food deprivation during the daytime (i.e., in the nearly

FIG. 2. Effect of varying fasting periods on basal (t=0) blood glucose (●), plasma IRI (○) and FFA (▲) levels (mean $\pm$ SEM), n=6.

ad lib condition) or after extended fasting periods of 12, 18 and 24 hr (see Fig. 1). A saline infusion of 0.9% (w/v) NaCl after 6 hr served as a control. The glucose infusions were given at random on separate days with intervals of 2 days after the 6 and 12 hr fasting periods. An interval of at least 4 days was used after 18 and 24 hr of fasting in order to prevent disturbance on the following experiments. Baseline values of FFA, glucose and insulin were established in blood samples taken at -10 and 0 min (start of the glucose infusion) and the response of those by sampling at 1, 3, 5, 10, 15, 20, 25 and 30 min.

Chemical Determinations

Blood glucose was measured by the ferricyanide method of Hoffman with a Technicon Autoanalyzer on 0.05 ml whole blood from each 0.5 ml sample. The remaining 0.45 ml blood was centrifuged at 4°C and 0.15 ml of the plasma was used for the free fatty (FFA) assay and the remaining plasma was stored at -30°C for the insulin assay.

Plasma FFA was determined with the autoanalyzer according to the method of Antonis [1] based on the measurements of the chloroform soluble copper salts of the longchain fatty acids. The method was adapted to 0.15 ml plasma.

Rat-specific plasma immunoreactive insulin (IRI) was determined by means of a radioimmunoassay kit (NOVO). Guinea pig serum M8309 served as antiserum for the insulin assay. Duplicate assays were performed on 25 μ l plasma samples. The bound and free 125 I-labeled insulin was separated by means of a polyethylene glycol solution (23.75% w/w). The coefficient of variation of the immunoassay was <8%.

Statistical Analysis and Calculations

Correlations were calculated with Kendall's rank correlation method. Differences were tested for statistical significance with paired *t*-test, *p*<0.05 (two tailed) being considered significant. Results are stated as mean $\pm$ SEM. Responses were quantified by measuring integrated areas below (FFA) or above the baseline (glucose, insulin) with a

FIG. 3. Effect of fasting periods of 6, 12, 18 and 24 hr on plasma IRI, FFA and blood glucose responses to intravenous glucose infusion over 20 min (10 mg/min), $n=6$, mean $\pm$ SEM.

Hewlett Packard 9874A Digitizer, connected with a 9835A HP computer.

RESULTS

Feeding Behavior

During ad lib conditions rats ate most of their food in the night (Fig. 1). During the first 6 hr of the light phase there was almost no feeding activity, whereas feeding increased gradually during the latter half of the light phase. Feeding activity was bimodally distributed over the dark phase with peaks near the beginning and near the end of the dark phase. Therefore, 6 hr of food deprivation (i.e., the first 6 hr of the light phase) can be considered as similar to the ad lib condition, and 12, 18 and 24 hr of food deprivation thereby imposing small, medium and long fasting periods.

Basal Values in the Plasma

Basal glucose did not differ significantly after 6, 12 or 18 hr of fasting averaging around 6 mmol/l (Fig. 2). After 24 hr of fasting basal glucose dropped significantly below the values of the latter conditions, reaching a level of 5.2 ± 0.1 mmol/l ($p < 0.05$). Basal immunoreactive insulin (IRI) decreased gradually from 40 ± 3 mU/l in the ad lib condition to 27 ± 3 , 21 ± 2 and 17 ± 2 mU/l (mean $\pm$ SEM) after fasting periods of 12, 18 and 24 hr, respectively. The difference became significant by 12 hr ($p < 0.01$). There was a significant negative correlation between basal plasma IRI and fasting time ($p < 0.01$, $\tau = 0.49$). Basal plasma FFA increased gradually during fasting from 0.21 ± 0.02 to 0.27 ± 0.02 , 0.35 ± 0.03

and 0.40 ± 0.02 mEq/l. The change was significant by 12 hr ($p < 0.05$). There was a significant positive correlation between plasma FFA and fasting time ($p < 0.01$, $\tau = 0.63$).

Responses to Intravenous Glucose

Blood glucose concentration increased immediately upon start of the glucose infusion, the most rapid rise occurring during the initial 5 min with a progressively less rapid increase which lasted until the end of the infusion 20 min later (Fig. 3). Integrated blood glucose responses increased with increasing fasting times (Fig. 4). Compared with the ad lib condition significant difference was observed already after 12 hr of fasting ($p < 0.05$). Coincident with the rapid rise of blood glucose plasma IRI markedly rose and was evident at the first minute (Fig. 3). The 1 min values reflected the initial phase of a typical biphasic pattern of responding. A major secondary rise of IRI appeared between 3 and 5 min and lasted until the infusion was terminated at 20 min. Plasma IRI rapidly declined to baseline values. Integrated plasma IRI responses over the 20 min decreased with increasing fasting times ($p < 0.02$, $\tau = 0.37$) (Fig. 4). Plasma insulin responses were slightly decreased after 12 hr ($p < 0.1$), compared with the ad lib condition. Significant differences were observed after 18 ($p < 0.05$) and 24 hr of fasting ($p < 0.01$). The responsiveness of the B-cell for glucose increments can be ascertained by the calculation of the insulinogenic indexes, i.e., quotient of integrated insulin response and glucose response. We obtained the following indexes: 1.06, 0.85, 0.67 and 0.33 for the 6 hr, 12 hr, 18 hr and 24 hr of fasting, respectively.

FIG. 4. Integrated responses of blood glucose (●), plasma IRI (○) and FFA (▲) after fasting periods of 6, 12, 18, and 24 hr. Responses were quantified by measuring integrated areas below (FFA) and above the baseline (glucose, insulin) over the 20 min infusion period (Arbitrary Units (A.U.), mean $\pm$ SEM), $n=6$.

FFA levels began decreasing after the first phase of insulin release and the decrease was greater with increased fasting time ($p < 0.05$, $r = 0.29$) (Fig. 3). The first significant difference was observed after 12 hr of fasting ($p < 0.05$). The antilipolytic effect was assessed by calculating an antilipolytic index, i.e., quotient of integrated FFA responses and insulin responses. We obtained the following indexes: 0.55, 1.15, 1.57 and 1.86 for the 6, 12, 18 and 24 hr of fasting, respectively. The saline control infusions did not show significant changes in each of the measured variables.

DISCUSSION

The present study shows that the responsiveness of insulin secreting B-cells for glucose is decreased with increasing fasting times. This holds for both the first phase of insulin release as well as for insulin secretion during the second phase. This decrease in responsiveness to glucose was especially prominent after 18 and 24 hr of fasting but was already manifest after 12 hr fasting.

Since during fasting the B-cells are not stimulated by the effects of food intake, basal plasma IRI levels gradually decreased. It is likely that this is caused not only by mechanisms which remove insulin from the circulation, but also by a decreased responsiveness of the B-cells [4, 10, 25]. We focussed our attention on basal insulin levels which are supposed to be a function of secretory responsiveness [8]. The close relationship between responsiveness (the insulinogenic index) and basal insulin levels is also clear from this study. Since insulin has a strong antilipolytic action and is a potent stimulus for uptake of FFA by tissues, especially in adipose tissue [6], the drop in basal insulin level after a fast should allow elevation of the FFA concentration. After fasting the

basal rate of lipolysis is markedly increased [6,7]. This is reflected in the observed increased basal FFA levels during fasting. In contrast herewith is the increase of the antilipolytic index during prolonged fasting. In vitro studies may explain this since after fasting adipose tissue is more sensitive for insulin binding [11,24]. While in vitro insulin-stimulated glucose uptake in fat cells is reduced after fasting [11,24] an increased uptake of FFA is possible. After a meal a parallel reduction was seen in plasma FFA and plasma glycerol [12] which may indicate that mainly lipolytic activity is affected by insulin and not the uptake of FFA in the tissues.

After fasting basal release of glycerol and FFA from fat cells in femoral regions is more sensitive for the inhibiting influence of insulin [3]. The increased sensitivity for the antilipolytic action of insulin after fasting may play an important role in the causation of the phenomenon. This new finding holds a paradox at first sight because the increased antilipolytic effect of insulin is related to the higher rate of basal lipolysis and a lower rate of glucose oxidation. It is conceivable, however, that this effect should prevent the development of excessive lipolysis and subsequent ketoacidosis. An elevation of basal FFA levels resulting in more FFA utilization in tissues [7,9] permits a lower glucose consumption in which the reciprocal relationship between glucose and FFA metabolism, known as the glucose-fatty acid cycle may play an additional role [14,16]. These processes in which the decreased responsiveness of insulin secretion plays a key function may contribute to prolonging the availability of glucose for the central nervous system by conserving liver glycogen. The sharp drop of basal glucose between 18 and 24 hr of fasting may indicate that the glycogen stores are depleted to a critical level. Evidence exists that during that time gluconeogenesis is activated to maintain glucose production on a low level [13]. During starvation glucagon plays an important role in the stimulation of gluconeogenesis [19]. The ability of muscle and other tissues to use fat as metabolic fuel during fasting also saves protein as a source for gluconeogenesis [5]. In summary, it is suggested that the decreased responsiveness of insulin secretion by the B-cell after moderate fasting periods results in a decrease of basal insulin. This in turn facilitates the conversion from glucose to FFA metabolism even when the first meals are missed, in order to save the glycogen stores as long as possible as fuel for the central nervous system and still support basic energy requiring processes adequately. Although the decrease in plasma insulin may allow increased lipolysis, excessive lipolysis is prevented by an increased antilipolytic action of insulin during fasting.

ACKNOWLEDGEMENTS

The authors express their thanks to Professor S. C. Woods and Dr. A. B. Steffens for discussing the manuscript, to F. Klatter, C. Meihuizen, T. M. Hofland and J. Westerhoff for technical assistance, and to Mrs. J. Poelstra-Hiddinga for typing the manuscript. This investigation was partly supported by the Foundation for Medical Research (FUNGO), which is subsidized by the Dutch Organization for the Advancement of Pure Research (ZWO).

REFERENCES

1. Antonis, A. Semiautomated method for colorimetric determination of plasma free fatty acids. *J Lipid Res* 6: 307-312, 1965.
2. Armstrong, S., J. Clarke and G. Coleman. Light dark variation in laboratory rat stomach and small intestine content. *Physiol Behav* 21: 785-788, 1978.
3. Arner, P., J. Bolinder, P. Engfeldt and P. Ostman. The antilipolytic effect of insulin in human adipose tissue in obesity, diabetes mellitus, hyperinsulinemia and starvation. *Metabolism* 30: 753-760, 1981.
4. Bouman, P. R., G. H. J. Wolters and W. Konijnendijk. Insulin secretion and cyclic adenosine 3', 5'-monophosphate levels in pancreatic islets of fed and fasted rats. *Diabetes* 28: 132-140, 1979.
5. Cahill, G. F. Starvation in man. *N Engl J Med* 282: 668-675, 1970.
6. Carlson, L. A., J. Boberg and B. Hogstedt. Some physiological and chemical implications of lipid mobilization from adipose tissue. In: *Handbook of Physiology. Adipose Tissue*, sect 5, Chapt 63, edited by A. E. Renold and G. F. Cahill. Washington, DC: Am Physiol Soc, 1965, pp. 625-644.
7. Ensink, J. W. and R. H. Williams. Hormonal and non hormonal factors modifying man's response to insulin. In: *Handbook of Physiology. Endocrine Pancreas*, sect 7, chapt 38, edited by R. O. Greep and E. B. Astwood. Washington, DC: Am Physiol Soc, 1972, pp. 665-684.
8. Goodner, C. J. and D. Porte. Determinants of basal islet secretion in man. In: *Handbook of Physiology. Endocrine Pancreas*, sect 7, chapt 38, edited by R. O. Greep and E. B. Astwood. Washington, DC: Am Physiol Soc, 1972, pp. 597-609.
9. Hagenfeldt, L. and J. Wahren. Metabolism of free fatty acids and ketone bodies in skeletal muscle. In: *Muscle Metabolism During Exercise*, edited by B. Pernow and B. Sattin. New York: Plenum Press, 1971, pp. 153-163.
10. Malaisse, W. J., F. Malaisse-Lagae and P. H. Wright. Effect of fasting upon insulin secretion in the rat. *Am J Physiol* 213: 843-848, 1967.
11. Olefski, J. M. Effects of fasting on insulin binding, glucose transport and glucose oxidation in isolated rat adipocytes. *J Clin Invest* 58: 1450-1460, 1976.
12. Owen, O. E., M. A. Mazzoli, G. Boden, M. Patel, G. A. Reichard, V. Trapp, C. R. Shuman and P. Felig. Substrate, hormone and temperature responses in males and females to a common breakfast. *Metabolism* 29: 511-523, 1980.
13. Palou, A., X. Remesar, L. I. Arola, E. Herrera and M. Alemany. Metabolic effects of short term food deprivation in the rat. *Horm Metab Res* 13: 326-330, 1981.
14. Pelkonen, R., T. A. Miettinen, M. R. Taskinen and E. A. Nikkila. Effect of acute plasma glycerol, triglyceride and FFA levels on glucose utilization and plasma insulin. *Diabetes* 17: 76-82, 1968.
15. Peret, J., M. Macaire and M. Chanez. Utilization and circadian rhythms of hepatic glycogen, plasma corticosteron and insulin in rats. *J Nutr* 103: 866-974, 1973.
16. Randle, P. J., P. B. Garland and C. N. Hales. The glucose fatty acid cycle and diabetes mellitus. *Ciba Found Colloq Endocrinol* 15: 192-212, 1964.
17. Steffens, A. B. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol Behav* 4: 833-836, 1969.
18. Steffens, A. B., G. J. Mogenson and J. A. F. Stevenson. Blood glucose insulin and free fatty acids after stimulation and lesions of the hypothalamus. *Am J Physiol* 222: 1446-1452, 1972.
19. Steffens, A. B. and J. H. Strubbe. CNS regulation of glucagon secretion. In: *CNS Regulation of Carbohydrate Metabolism, Vol 10: Advances in Metabolic Disorders*, edited by A. J. Szabo. New York: Academic Press, 1983, pp. 221-252.
20. Strubbe, J. H. A small swivel joint for infusion of freely moving animals. *Physiol Behav* 12: 317-319, 1969.
21. Strubbe, J. H. and A. B. Steffens. Rapid insulin release after ingestion of a meal in the unanesthetized rat. *Am J Physiol* 229: 1019-1022, 1975.
22. Strubbe, J. H. and P. van Wachem. Insulin secretion by the transplanted neonatal pancreas during food intake in fasted and fed rats. *Diabetologia* 20: 228-236, 1981.
23. Vonk, R. J., A. B. D. van Doorn and J. H. Strubbe. Bile secretion and composition in the freely moving unanesthetized rat with a permanent biliary drainage: influence of food intake on bile flow. *Clin Sci Mol Med* 55: 253-259, 1978.
24. Wierenga, T. and H. M. J. Krans. Reduced glucose transport and increased binding of insulin in adipocytes from diabetic and fasted rats. *Biochim Biophys Acta* 538: 563-570, 1978.
25. Wolters, G. H. J., W. Konijnendijk and P. R. Bouman. Effects of fasting on insulin secretion, islet glucose metabolism and the cyclic adenosine 3', 5'-monophosphate content of rat pancreatic islets in vitro. *Diabetes* 26: 530-537, 1977.