Effect of Glucose Stimulation on ^{45}Calcium Uptake and Total Calcium Content of Pancreatic Islets of Fed and Fasted Rats and Obese Hyperglycemic Mice

G. H. J. WOLTERS, J. B. WIEGMAN, AND W. KONIJNENDIJK

SUMMARY

Effects of fasting on glucose-induced changes of various calcium parameters were investigated. The total calcium content of islets of fed rats and obese mice, determined by fluorometric microanalysis, amounted to 575 ± 25 and 600 ± 75 pmol/μg DNA, respectively. Fasting for 24 or 72 h had no effect on the calcium content of rat islets, but increased that of mice by 30%. Glucose stimulation did not alter their total calcium content. The amount of ^{45}Ca (as percentage of total islet calcium content) taken up over 30 min by fed mouse islets at 2.5, 10, and 15 mM glucose was equivalent to 32%, 41%, and 44% and by fed rat islets to 25%, 31%, and 34%, respectively. The net uptake of fed rat islets over 60 min at 2.5 and 15 mM glucose amounted to 36% and 61%. Fasting for 24 h inhibited insulin secretion over 30 min but not ^{45}Ca uptake, whereas both parameters were inhibited after fasting for 72 h. Insulin secretion of mouse islets was inhibited after 24 and 72 h of fasting, but ^{45}Ca uptake was not significantly affected.

Since glucose stimulates ^{45}Ca uptake without changing the total islet calcium content, it must represent ^{45}Ca–^{40}Ca exchange. Stimulation of insulin secretion of rat islets is associated with an increased calcium influx rate and increased exchangeable calcium pool(s) size. Calcium pool(s) size seems not to be altered by 24 h of fasting; however, 72 h of fasting tends to decrease the pool(s) size. The inhibition of insulin secretion induced by 24 h of fasting is not due to an altered calcium uptake, but it may be due to a modification of intracellular handling of calcium. DIABETES 32:124–129, February 1983.

Fasting causes a decrease of the insulin secretory response to glucose stimulation in vivo as well as in vitro[1–6] and seems a particularly promising model for the study of impaired insulin secretion. The physiologic and biochemical processes that occur in the endocrine pancreas during fasting are not well understood. Abnormal glucose metabolism[7,8] or decreased cyclic AMP generation[4,5,9,10] have been considered as possible causes of diminished insulin secretory response. In addition, calcium, an important regulator of the insulin secretory process, seems to be involved in the fasting-induced secretory disturbance.

Recently, we demonstrated that fasting causes a strong decrease of a histochemically detectable calcium fraction (mobile calcium) of islets in situ without altering the total calcium content of islet tissue.[11] Furthermore, it has been reported that fasting for 48 h decreases the ^{45}Ca uptake of rat islets.[7] In contrast, however, it has been observed that fasting for 24 or 72 h increases the ^{45}Ca uptake of islets of obese mice.[12] Since in the last two studies only the ^{45}Ca content and not the total islet calcium content was measured, differences in the islet calcium content and/or turnover rate in both species may have contributed to these contrasting results. We therefore investigated the effect of fasting on ^{45}Ca uptake and the total calcium content of islets, using a direct fluorometric microanalysis for determination of the latter. In ^{45}Ca uptake studies, washing procedures of questionable validity and reproducibility are sometimes used to remove extracellularly trapped calcium. In the present study we carefully removed extracellular calcium without loss of intracellular calcium by using a rapid microfiltration procedure.[13]

MATERIALS AND METHODS

Isolation of islets. Pancreatic islets were isolated from male Wistar rats (350 g) and obese hyperglycemic mice (both sexes, 6–10 mo, 60 g) originally derived from the Jackson Memorial Laboratory (Bar Harbor, Maine) and bred since 1958 at the Department of Genetics, University of Nijmegen, The Netherlands. The animals used were either fed, fasted 24 h, or fasted 72 h with free access to drinking water. In each experimental session islets were isolated with partially

From the Department of Experimental Endocrinology, University of Groningen, 1 Bloemsingel, 9713 BZ Groningen, The Netherlands.
Address reprint requests to Dr. G. H. J. Wolters at the above address.
Received for publication 5 May 1982 and in revised form 15 September 1982.

TABLE 1
Effect of glucose stimulation on total calcium content and uptake of ^{45}Ca over 5 min of islets of fed, 24-h-fasted, and 72-h-fasted rats

	Fed	24-h-fasted	72-h-fasted
Glucose			
2.5 mM			
Total calcium content	599 ± 39	498 ± 46	500 ± 47
^{45}Ca uptake	114 ± 10	103 ± 12	99 ± 11
Uptake as percentage of total	20 ± 1	21 ± 2	20 ± 1
15 mM			
Total calcium content	538 ± 40	569 ± 58	511 ± 43
^{45}Ca uptake	166 ± 17*	165 ± 19†	147 ± 15‡
Uptake as percentage of total	31 ± 2	29 ± 1	29 ± 2

Results expressed as pmol Ca/μg DNA, mean ± SEM of 11 separate experiments. DNA content per islet, 63 ± 1 ng (N = 195). Glucose-induced increment of the ^{45}Ca uptake: *,†P < 0.001; ‡P < 0.005 (Student's *t* test for paired data).

purified collagenase[4] from pancreata of two rats or three mice of each nutritional category. Pools of 700 rat islets or 350 mouse islets of each category were collected.

Incubation and ^{45}Ca uptake of islets. The islet pools were preincubated for 15 min at 37°C in Krebs-Ringer bicarbonate buffer containing 2.5 mg/ml bovine serum albumin, 2.5 mM $CaCl_2$, and 2.5 mM glucose and gassed with O_2 + CO_2 (95:5). Batches of 30 rat islets or 15 mouse islets were selected and transferred to vessels containing 1 ml of buffer (2.5 mM glucose), which were placed in a shaking incubator. The incubation was started by the addition of 1 ml of buffer containing 20 μCi $^{45}CaCl_2$ (The Radiochemical Centre, Amersham, Bucks, United Kingdom) to a final concentration of 2.5 mM calcium and glucose to a final concentration of 2.5, 10, or 15 mM.

Measurement of the total calcium content of islets. At the end of the incubation the islets were rapidly filtered, washed (10 s), and sonicated in 100 μl of water. Samples were taken for the determination of DNA,[14] and the calcium content was determined by a fluorometric micromethod as described recently.[13]

Measurement of ^{45}Ca content of islets. After determination of the total calcium content, the same samples were transferred to scintillation vials. The net uptake of calcium from the medium was calculated from the ^{45}Ca uptake and the specific activity of ^{45}Ca in the medium. The results were expressed as pmol Ca/μg DNA.

Insulin secretion. Insulin secretion of islets of fed and fasted rats and obese mice was measured simultaneously with the ^{45}Ca uptake over 30 min. The insulin content of the incubation medium at 0 and 30 min was determined by radioimmunoassay using guinea pig anti-rat insulin antiserum and rat insulin as a standard. Samples of rat and mouse insulin diluted parallel in this system. Free and bound insulin in the assay were separated by polyethylene glycol precipitation.[15]

RESULTS

^{45}Ca uptake and total calcium content of fed and fasted rat islets exposed for 5 min to 2.5 or 15 mM glucose. Glucose (15 mM) enhances the net uptake of Ca by islets of fed rats over 5 min by about 50% relative to the basal uptake (Table 1). Total calcium content was not significantly affected by raising the glucose concentration. Fasting for 24 or 72 h did not alter either the total calcium content of the islets or the basal and glucose-stimulated Ca uptake. The percentage of labeled calcium in islets of fed and fasted rats at 2.5 and 15 mM glucose also did not differ.

^{45}Ca uptake and total calcium content of fed and fasted rat islets exposed for 30 min to 2.5, 10, and 15 mM glucose. Elevating the glucose concentration from 2.5 to 10 or 15 mM glucose increased the net uptake of Ca of islets of fed rats by 40% and 50%, respectively (Table 2). The basal net uptake of Ca was not significantly affected by fasting for

TABLE 2
Effect of glucose stimulation on total calcium content and net uptake of ^{45}Ca over 30 min of islets of fed, 24-h-fasted, and 72-h-fasted rats

	Fed	24-h-fasted	72-h-fasted
Glucose			
2.5 mM			
Total calcium content	586 ± 65	575 ± 49	551 ± 20
^{45}Ca uptake	135 ± 6	123 ± 8	130 ± 8
Uptake as percentage of total	25 ± 2	20 ± 2	23 ± 2
10 mM			
Total calcium content	630 ± 50	585 ± 62	573 ± 36
^{45}Ca uptake	190 ± 9*	182 ± 13†	152 ± 6‡
Uptake as percentage of total	31 ± 1	32 ± 2	28 ± 2
15 mM			
Total calcium content	617 ± 55	595 ± 48	558 ± 42
^{45}Ca uptake	203 ± 10§	207 ± 13‖	171 ± 4¶
Uptake as percentage of total	34 ± 2	34 ± 2	31 ± 2

Results expressed as pmol Ca/μg DNA, mean ± SEM of eight separate experiments. DNA content per islet, 64 ± 1 ng (N = 288). Glucose-induced increment of the ^{45}Ca uptake: *,†,§,‖,¶P < 0.001; ‡P < 0.05; * versus §,P > 0.05; † versus ‖, P < 0.001; ‡ versus ¶, P < 0.05. * versus ‡, P < 0.02; § versus ¶, P < 0.05 (Student's *t* test for paired data).

TABLE 3
Effect of glucose stimulation on total calcium content and net uptake of ^{45}Ca over 60 min of islets of fed, 24-h-fasted, and 72-h-fasted rats

	Fed	24-h-fasted	72-h-fasted
Glucose			
2.5 mM			
Total calcium content	526 ± 37	534 ± 49	496 ± 32
^{45}Ca uptake	188 ± 15	196 ± 28	170 ± 19
Uptake as percentage of total	36 ± 2	36 ± 2	35 ± 2
15 mM			
Total calcium content	536 ± 27	557 ± 43	560 ± 51
^{45}Ca uptake	325 ± 20*	334 ± 33†	293 ± 39‡
Uptake as percentage of total	61 ± 3	60 ± 3	52 ± 3

Results expressed as pmol Ca/μg DNA, mean ± SEM of 11 separate experiments. DNA content per islet, 63 ± 1 ng (N = 191). Glucose-induced increment of the ^{45}Ca uptake: *,†$P < 0.001$; ‡$P < 0.005$ (Student's *t* test for paired data).

24 or 72 h, and the glucose-induced increment of the net uptake of Ca was not significantly influenced by 24 h of fasting. Fasting for 72 h depressed the Ca uptake at 10 and 15 mM glucose. The total calcium content of fed, 24-h-fasted, and 72-h-fasted islets did not differ after 30 min of incubation at 2.5 mM glucose and was not affected by raising the glucose concentration to 10 or 15 mM. The percentage of labeled Ca in the islets is not altered by fasting.

^{45}Ca uptake and total calcium content of fed and fasted rat islets exposed for 60 min to 2.5 or 15 mM glucose. In islets of fed rats 15 mM glucose stimulated the net uptake of Ca during 60 min by 70% relative to 2.5 mM glucose (Table 3). In 24-h-fasted islets identical results were obtained. Fasting for 72 h tends to inhibit the Ca uptake at 15 mM glucose. The percentage of labeled calcium in the islets of 72-h-fasted rats is significantly lower ($P = 0.025$, Student's paired *t* test) than in islets of fed rats, which indicates that 72 h of fasting altered islet calcium handling. In spite of a net Ca uptake ranging from 52% to 61% of the total islet calcium content at 15 mM glucose, total islet calcium content was not significantly increased in either of the three categories of islets in response to 60 min of glucose stimulation.

^{45}Ca uptake and total calcium content of islets of fed and fasted obese hyperglycemic mice after exposure for 30 min to 2.5, 10, or 15 mM glucose. After 30 min of incubation at 2.5 mM glucose the total calcium content of islets of 24-h- and 72-h-fasted mice appeared to be about 30% higher than that of islets of fed mice. The total calcium content of fed and fasted islets was not significantly affected by an increase of the glucose concentration to 10 or 15 mM, and the difference in calcium content of fed and fasted islets was maintained (Table 4). The amount of Ca taken up at 2.5 mM glucose was not altered by fasting. Glucose (10 mM) enhanced the net uptake of Ca by fed islets by 30%. Increasing the glucose concentration to 15 mM did not further augment the Ca uptake. The amounts of Ca taken up by 24-h- and 72-h-fasted islets were not significantly different from that of fed islets. However, due to the higher islet calcium content, the percentage of exogenous calcium in fasted islets was lower than in fed islets.

Insulin secretion by islets of fed and fasted rats and obese hyperglycemic mice. In the experiments shown in Tables 2 and 4, insulin secretion over 30 min was also measured. At 15 mM glucose, fed rat islets secreted twice as much insulin as at 10 mM, but mouse islets secreted only 40% more (Figure 1). Fasting for 24 and 72 h did not affect the basal insulin secretion in rat as well as in mouse islets. Insulin secretion of islets of 24-h- and 72-h-fasted rats incubated at 10 mM glucose was inhibited by 69% and 90% and at 15 mM glucose by 32% and 78%, respectively. Insulin secretion of islets of obese mice fasted for 24 or 72 h incubated at 10 mM glucose was inhibited by 42% and 67% and at 15 mM glucose by 36% and 60%, respectively.

Figures 2 and 3 show the relationship between 10- and15-

TABLE 4
Effect of glucose stimulation on total calcium content and net uptake of ^{45}Ca over 30 min of islets of obese hyperglycemic mice

	Fed	24-h-fasted	72-h-fasted
Glucose			
2.5 mM			
Total calcium content	588 ± 80	773 ± 65	775 ± 53
^{45}Ca uptake	190 ± 10	158 ± 13	155 ± 18
Uptake as percentage of total	32 ± 3	23 ± 3	22 ± 3
10 mM			
Total calcium content	628 ± 75	858 ± 93	800 ± 38
^{45}Ca uptake	253 ± 18*	240 ± 13†	213 ± 23‡
Uptake as percentage of total	41 ± 2	30 ± 2	27 ± 3
15 mM			
Total calcium content	580 ± 58	868 ± 95	785 ± 48
^{45}Ca uptake	253 ± 18§	258 ± 13‖	228 ± 18¶
Uptake as percentage of total	44 ± 4	32 ± 3	30 ± 3

Results expressed as pmol Ca/μg DNA, mean ± SEM of 7–8 separate experiments. DNA content per islet, 128 ± 2 ng (N = 374). Glucose-induced increment of the ^{45}Ca uptake: *$P < 0.02$; †$P < 0.01$; ‡,§,‖$P < 0.005$; ¶$P < 0.001$; * versus §, † versus ‖, ‡ versus ¶, $P > 0.05$ (Student's *t* test for paired data).

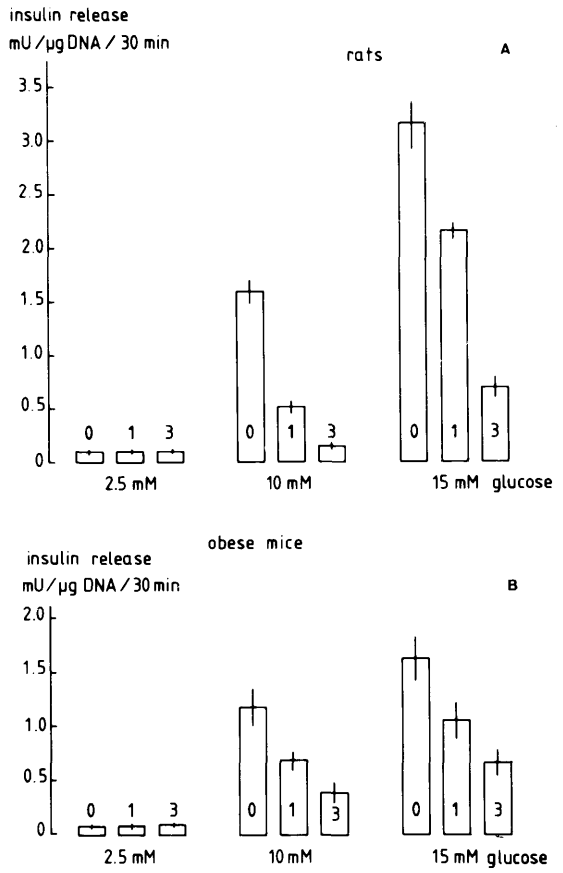

FIGURE 1. Effect of fasting on insulin secretion of islets of rats (A) and obese hyperglycemic mice (B) at 2.5, 10, and 15 mM glucose. Fed (0); 24-h-fasted (1); 72-h-fasted (3). Mean ± SEM of eight separate experiments.

mM glucose-induced increase of the ^{45}Ca uptake and insulin secretion. Twenty-four hours of fasting decreases insulin secretion in both rat and mouse islets, but the ^{45}Ca uptake tends to be even higher. Fasting for 72 h inhibits the 10- and 15-mM glucose-induced insulin secretion in rat as well as in mouse islets, but decreases only the ^{45}Ca uptake of rat islets.

DISCUSSION

Effects of glucose stimulation on the total calcium content of fed rat islets. The glucose-induced ^{45}Ca uptake at 15 mM glucose amounted to 137 pmol/μg DNA at 60 min (25% of the total islet calcium content). If the calcium content was increased by the same amount, it should have been detected. Thus, the stimulated ^{45}Ca uptake apparently reflects an increased calcium exchange with the medium, resulting in an unmodified but more heavily labeled total islet calcium pool. This is in accordance with Andersson et al.,[16] who also could not demonstrate a significant effect of glucose on the total calcium content of mouse islets incubated for 120 min. They also found a relatively small amount of ^{45}Ca taken up in response to glucose. In contrast, Ribes et al.[17] claimed a rapid and large glucose-induced augmentation of the total islet calcium content. Their results, however, were not based on direct measurement of total calcium but on glucose-induced changes in ^{45}Ca content of islets cultured with ^{45}Ca until isotopic equilibrium. The changes in ^{45}Ca content were assumed to reflect changes in the total calcium content of the islets, which may not be the case as suggested by our present findings.

^{45}Ca influx rate of islets of fed rats. The ^{45}Ca uptake by rat islets over the initial 5 min reflects more or less an unidirectional flux.[18] The 15-mM glucose-induced increment of the ^{45}Ca influx per 5 min (53 ± 11 pmol/μg DNA) is equivalent to 11% of the total calcium content, or 2.2%/min, which is much less than the increase of 10%/min as calculated by Ribes et al.[17] However, expressed per islet, we found an uptake comparable with that of Ribes et al. (0.67 and 0.44 pmol/islet/min, respectively).

The calcium influx rate is high, and since the total calcium content does not increase islet cells must possess a highly effective process for limiting the intracellular calcium content by an efflux mechanism capable of turnover at a high rate.

Effect of glucose stimulation on the net uptake of ^{45}Ca over 30 and 60 min. The 15 mM glucose-induced increment of the ^{45}Ca uptake of islets of fed rats over 5 and 30 min amounted to 11 ± 1% and 9 ± 1%, respectively, and after 60 min to 25 ± 3% of the total islet calcium content. These findings suggest that the glucose-sensitive exchangeable calcium pool is constant in the period 5–30 min, but increases between 30 and 60 min. The time kinetics of the ^{45}Ca uptake of rat islets in our experiments are in contrast with those of Naber et al.[19] and Hellman et al.,[20] who observed a more or less constant difference between ^{45}Ca uptake at low and high glucose from 30 min up to at least 70 or 90 min. We have no explanation for this discrepancy, but it might be due to methodologic differences. The increase of the ^{45}Ca-exchangeable calcium pool after 30 min of glucose stimulation may be related to the same mechanism that also increases the histochemically detectable mobile calcium content of the islets in this period.[21]

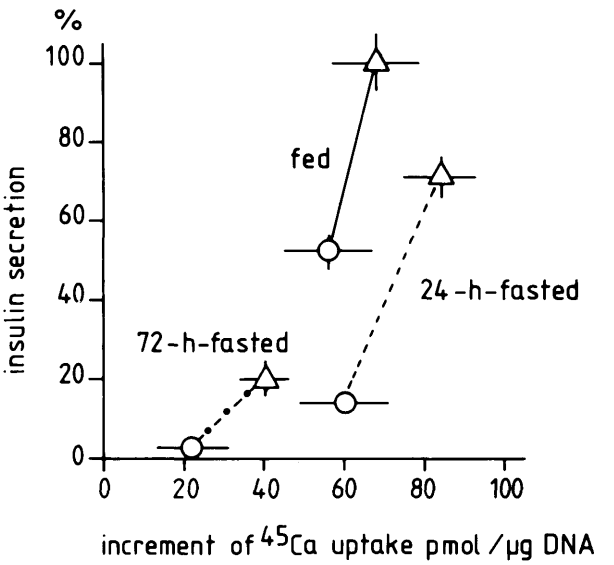

FIGURE 2. Relationship between insulin output above basal value induced by 10 (○) and 15 mM (△) glucose and the corresponding values for glucose-induced increment of ^{45}Ca in islets of fed and fasted rats. Insulin secretion at 10 and 15 mM glucose of islets of fed and fasted rats is expressed as percentage of the secretion of islets of fed rats at 15 mM glucose (2.9 ± 0.2 mU/μg DNA/30 min). ^{45}Ca uptake fed versus 72-h-fasted both at 10 mM and 15 mM glucose, $P < 0.05$. Mean ± SEM of eight separate experiments.

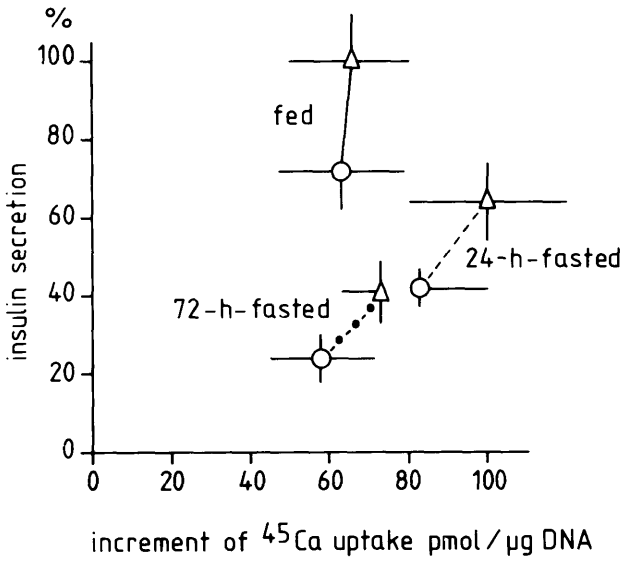

FIGURE 3. Relationship between insulin output above basal value induced by 10 (○) and 15 mM (△) glucose and the corresponding values for glucose-induced increment of ^{45}Ca in islets of fed and fasted mice. Insulin secretion at 10 and 15 mM glucose of islets of fed and fasted mice is expressed as percentage of the secretion of islets of fed mice at 15 mM glucose (1.6 ± 0.2 mU/µg DNA/30 min). ^{45}Ca uptake fed versus 24-h-fasted at 10 mM glucose, $P > 0.05$; at 15 mM glucose, $P < 0.001$. Mean ± SEM of eight separate experiments.

Effect of fasting on ^{45}Ca uptake and total calcium content of rat islets. Fasting for 24 or 72 h has no influence on the total islet calcium content, which confirms our previous observation.[11] The glucose-stimulated net uptake of ^{45}Ca over 5, 30, and 60 min is not affected by 24 h of fasting; however, fasting for 72 h inhibited the glucose-stimulated net uptake of ^{45}Ca over 30 min. No significant effect is seen after 60 min. It is known that glucose metabolism is depressed after 72 h of fasting but not after 24 h.[4,7,22] The decreased ^{45}Ca uptake of islets of 72-h-fasted rats may therefore be an attendant effect of their decreased glucose metabolism. Our results are at variance with those of Levy et al.,[7] who observed an inhibition of 50% of the ^{45}Ca uptake in islets of 48-h-fasted rats even after 90 min at 16.7 mM glucose. However, in their study the islets were washed extensively, which could result in a loss of intracellular calcium.[13]

Total calcium content and ^{45}Ca uptake of islets of fed and fasted obese hyperglycemic mice. Islets of fed obese hyperglycemic mice contain similar amounts of calcium as islets of fed rats. However, fasting has no effect on the total calcium content of rat islets whereas it increases the calcium content of mouse islets by 30%. The B-cells contain several calcium pools; one such pool, the secretory granules, contains the highest calcium concentration.[23] The B-cells of obese hyperglycemic mice are poorly granulated. Fasting for 24–72 h increases the granules content of the B-cells 2–3 times, but also strongly increases the histochemically detectable calcium content of the islets.[24] This histochemical calcium fraction is believed to be mainly localized in the secretory granules as degranulation causes almost complete disappearance of this calcium fraction and decreases the total islet calcium content by 35%.[11] Fasting strongly decreases the histochemical calcium content of rat islets[11,25,26] without altering the granules content of the B-cells, which is in contrast to islets of obese mice. This indicates that the islet calcium metabolism of rats and obese mice is differently affected by fasting. The fasting-induced increase of the granules content of the B-cells of mouse islets may at least partially explain the increase of the calcium content of mouse islets.

The glucose-stimulated ^{45}Ca uptake by islets of fed mice over 30 min was not significantly different whether the islets were stimulated with 10 or 15 mM glucose (Figure 3). The amount of ^{45}Ca taken up at 10 and 15 mM glucose is not significantly affected by fasting, although the total calcium content of fasted islets is about 30% higher. In contrast, an increased ^{45}Ca uptake by fasted mouse islets was observed by Hellman et al.[12] on stimulation for 60 or 120 min. Possibly, if we would have extended the incubation for a prolonged time, our fasted islets with their higher calcium content also might have exchanged more ^{45}Ca than fed islets.

Relationship between ^{45}Ca net uptake and insulin secretion. Acute exposure of rat islets to glucose leads to a short-lived initial burst of insulin secretion followed by a rise in the secretion rate during at least 20–30 min.[1,27,28] However, in the period of increasing secretion rate, we did not observe an increasing glucose-stimulated uptake of ^{45}Ca in rat islets since the values after 5 and 30 min were similar. In addition, the secretion rate of our islets decreased considerably after 30 min (50% between 45 and 60 min),[25] whereas the stimulated ^{45}Ca net uptake (15 mM glucose) increased between 30 and 60 min to 25% of the total islet calcium content (compare Tables 2 and 3). When the data on glucose-stimulated ^{45}Ca uptake were compared with insulin output, it revealed that insulin secretion is not proportional to the ^{45}Ca uptake (Figure 2). The amount of insulin secreted by islets of fed rats at 10 mM glucose is associated with a ^{45}Ca net uptake of 55 pmol/µg DNA. At 15 mM glucose twice as much insulin is secreted as at 10 mM, but the ^{45}Ca net uptake is only slightly higher. In islets of 24-h-fasted rats the discrepancy is even more marked. Fasting for 24 h inhibits insulin secretion of rat islets but not the ^{45}Ca uptake, whereas after 72 h of fasting both parameters are inhibited. In mouse islets the inhibited secretory response is not associated with a decreased ^{45}Ca uptake even after 72 h of fasting (Figure 3). Thus, as shown in Figure 2 for rat islets and in Figure 3 for mouse islets, there is no correlation between insulin output and the ^{45}Ca uptake of the three nutritional categories. Fasting for 24 h decreases insulin secretion of rat islets without significantly affecting the ^{45}Ca uptake and the calcium content of the islets, suggesting an unaltered islet calcium metabolism. However, fasting for 24 or 72 h strongly decreased the histochemically detectable mobile calcium content of rat islets and its response to glucose.[11,25,26] Thus, fasting for 24 h apparently causes a disturbance of intracellular calcium handling, which does not find expression in an altered uptake of extracellular ^{45}Ca in response to glucose stimulation. A discrepancy in insulin output and ^{45}Ca uptake has also been observed when the cAMP content of islets was raised by the phosphodiesterase inhibitors 3-iso-butyl-1-methylxanthine, theophylline, and dibutyryl-cAMP.[29,30]

Glucose stimulation enhances the cAMP content of fed rat islets,[4,5] which mobilizes intracellular calcium[29,31,32] and reduces the uptake of ^{45}Ca into mitochondria[30] and probably in this way enlarges the pool of "trigger-Ca^{2+}." Fasting in-

hibits the cAMP response[4,5,10] and thus will interfere with the availability of intracellular Ca^{2+}. The inhibition of the secretory response as a result of short-term fasting is most likely due to a combined disturbance of cAMP generation and intracellular calcium handling.

ACKNOWLEDGMENTS

We thank J. Pleiter for preparing the figures and Prof. Dr. P. R. Bouman for the fruitful discussions.

REFERENCES

[1] Bosboom, R. S., Zweens, J., and Bouman, P. R.: Effects of feeding and fasting on the insulin secretory response to glucose and sulfonylureas in intact rats and isolated perfused rat pancreas. Diabetologia *9*:243–50, 1973.

[2] Hahn, H.-J., and Fiedler, H.: Investigations on isolated islets in vitro. Influence of food deprivation and glucose refeeding in vitro on insulin secretion. Acta Diabetol. Lat. *11*:457–67, 1974.

[3] Malaisse, W. J., Malaisse-Lagae, F., and Wright, P. H.: Effect of fasting upon insulin secretion in the rat. Am. J. Physiol. *213*:843–48, 1967.

[4] Wolters, G. H. J., Konijnendijk, W., and Bouman, P. R.: 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*:530–37, 1977.

[5] Bouman, P. R., Wolters, G. H. J., and Konijnendijk, W.: Insulin secretion and cyclic adenosine 3′,5′-monophosphate levels in pancreatic islets of fed and fasted rats. Time course and dose kinetics during glucose stimulation. Diabetes *28*:132–40, 1979.

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

[7] Levy, J., Herchuelz, A., Sener, A., and Malaisse, W. J.: The stimulus-secretion coupling of glucose-induced insulin release. Fasting: a model for altered glucose recognition by the B-cell. Metabolism *25*:583–91, 1976.

[8] Malaisse, W. J., Sener, A., and Levy, J.: The stimulus-secretion coupling of glucose-induced insulin release. Fasting-induced adaptation of key glycolytic enzymes in isolated islets. J. Biol. Chem. *251*:1731–37, 1976.

[9] Capito, K., and Hedeskov, C. J.: The effect of starvation on phosphodiesterase activity and the content of adenosine 3′:5′-cyclic monophosphate in isolated mouse pancreatic islets. Biochem. J. *142*:653–58, 1974.

[10] Rabinovitch, A., Grill, V., Renold, A. E., and Cerasi, E.: Insulin release and cyclic AMP accumulation in response to glucose in pancreatic islets of fed and starved rats. J. Clin. Invest. *58*:1209–16, 1976.

[11] Wolters, G. H. J., Pasma, A., Konijnendijk, W., and Boom, G.: Calcium, zinc and other elements in islet and exocrine tissue of the rat pancreas as measured by histochemical methods and electron-probe micro-analysis. Effects of fasting and tolbutamide. Histochemistry *62*:1–17, 1979.

[12] Hellman, B., Idahl, L.-Å., Lenzen, S., Sehlin, J., and Täljedal, I.-B.: Further studies on the relationship between insulin release and lanthanum-nondisplaceable $^{45}Ca^{2+}$ uptake by pancreatic islets: effects of fructose and starvation. Endocrinology *102*:1856–63, 1978.

[13] Wolters, G. H. J., Wiegman, J. B., and Konijnendijk, W.: The effect of glucose stimulation on ^{45}calcium uptake of rat pancreatic islets and their total calcium content as measured by a fluorometric micro-method. Diabetologia *22*:122–27, 1982.

[14] Kissane, J. M., and Robins, E.: The fluorometric measurement of deoxyribonucleic acid in animal tissue with special reference to the central nervous system. J. Biol. Chem. *233*:184–88, 1958.

[15] Desbuquois, B., and Aurbach, G. D.: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J. Clin. Endocrinol. *33*:732–38, 1971.

[16] Andersson, T., Berggren, P.-O., and Gylfe, E.: Effects of glucose on the calcium content of intact β-cells and cellular organelles. Ups. J. Med. Sci. *86*:165–70, 1981.

[17] Ribes, G., Siegel, E. G., Wollheim, C. B., Renold, A. E., and Sharp, G. W. G.: Rapid changes in calcium content of rat pancreatic islets in response to glucose. Diabetes *30*:52–55, 1981.

[18] Wollheim, C. B., Kikuchi, M., Renold, A. E., and Sharp, G. W. G.: Somatostatin- and epinephrine-induced modifications of $^{45}Ca^{++}$ fluxes and insulin release in rat pancreatic islets maintained in tissue culture. J. Clin. Invest. *60*:1165–73, 1977.

[19] Naber, S. P., McDaniel, M. L., and Lacy, P. E.: The effect of glucose on the acute uptake and efflux of calcium-45 in isolated rat islets. Endocrinology *101*:686–93, 1977.

[20] Hellman, B., Gylfe, E., Berggren, P.-O., Andersson, T., Abrahamsson, H., Rorsman, P., and Betsholtz, C.: Ca^{2+} transport in pancreatic β-cells during glucose stimulation of insulin secretion. Ups. J. Med. Sci. *85*:321–29, 1980.

[21] Wolters, G. H. J., Pasma, A., Konijnendijk, W., and Bouman, P. R.: Effects of calcium manipulation and glucose stimulation on a histochemically detectable mobile calcium fraction in isolated rat pancreatic islets. Histochemistry *66*:125–35, 1980.

[22] Zawalich, W. S., Dye, E. S., Pagliara, A. S., Rognstad, R., and Matschinsky, F. M.: Starvation diabetes in the rat: onset, recovery, and specificity of reduced responsiveness of pancreatic β-cells. Endocrinology *104*:1344–51, 1979.

[23] Howell, S. L., Montague, W., and Tyhurst, M.: Calcium distribution in islets of Langerhans: a study of calcium concentrations and of calcium accumulation in B cell organelles. J. Cell Sci. *19*:395–409, 1975.

[24] Wolters, G., Vonk, M., and Konijnendijk, W.: Histochemical calcium content of pancreatic islets and impaired insulin secretion. Neth. J. Med., 1982. Abstract. In press.

[25] Wolters, G. H. J., Pasma, A., and Konijnendijk, W.: Decreased response of mobile calcium in pancreatic islets of fasted rats to glucose stimulation and calcium manipulation. Diabetes, 1983. In press.

[26] Wolters, G. H. J., Konijnendijk, W., and Pasma, A.: Decreased response of mobile calcium in pancreatic islets of fasted rats to glucose. Diabetologia *19*:325–26, 1980.

[27] Lacy, P. E., Walker, M. M., and Finke, C. J.: Perifusion of isolated rat islets in vitro. Participation of the microtubular system in the biphasic release of insulin. Diabetes *21*:987–98, 1972.

[28] Ashby, J. P., and Shirling, D.: The priming effect of glucose on insulin secretion from isolated islets of Langerhans. Diabetologia *21*:230–34, 1981.

[29] Brisson, G. R., Malaisse-Lagae, F., and Malaisse, W. J.: The stimulus-secretion coupling of glucose-induced insulin release. VII. A proposed site of action for adenosine-3′,5′-cyclic monophosphate. J. Clin. Invest. *51*:232–41, 1972.

[30] Hahn, H.-J., Gylfe, E., and Hellman, B.: Calcium and pancreatic β-cell function. 7. Evidence for cyclic AMP-induced translocation of intracellular calcium. Biochim. Biophys. Acta *630*:425–32, 1980.

[31] Malaisse, W. J., Mahy, M., and Malaisse-Lagae, F.: Effect of sulfonylureas on calcium uptake and insulin secretion by islets of Langerhans. Arch. Int. Pharmacodyn. *192*:205–207, 1971.

[32] Brisson, G. R., and Malaisse, W. J.: The stimulus-secretion coupling of glucose-induced insulin release. XI. Effects of theophylline and epinephrine on ^{45}Ca efflux from perifused islets. Metabolism *22*:455–65, 1973.