CHAPTER 5

ABLATION OF CAPSAICIN-SENSITIVE AFFERENT NERVES AFFECTS INSULIN RESPONSE DURING AN INTRAVENOUS GLUCOSE TOLERANCE TEST BUT HAS NO EFFECT ON GLUCOSE TOLERANCE

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ABSTRACT

The role of sensory nerves in glucose tolerance was investigated in conscious Wistar rats treated neonatally with either the neurotoxin capsaicin or with vehicle. Intravenous glucose tolerance tests (IVGTT, 150, 300 and 450 mg in 30 min) were performed to measure glucose tolerance. Glucose, insulin and glucagon levels were measured. Higher glucose concentration resulted in a greater insulin response in both the experimental and the control animals. However, glucose-stimulated insulin secretion was attenuated in capsaicin-treated animals, while glucose levels did not differ between both groups. Glucagon levels did not differ between both groups. These results demonstrate that capsaicin-sensitive nerves are involved in glucose-stimulated insulin secretion, but are not required to regulate blood glucose levels. Moreover, it suggests that capsaicin-sensitive nerves could be involved in the regulation of insulin sensitivity. It could be hypothesized that sensory afferents could play a role in the aetiology, the onset, and development of pathologies where glucohomeostatic mechanisms are disturbed as is in type 2 diabetes.

1 INTRODUCTION

Insulin secretion is regulated both by autonomic nerves and by humoral factors. This is reflected by the rich innervations of pancreatic islets by parasympathetic, sympathetic and sensory nerves (1). Classical neurotransmitters harbored in cholinergic and adrenergic nerves are important in the control for islet hormone secretion and therefore for controlling glucose homeostasis (18, 24). Although the brain appears crucial for coordinating an adequate response to a glucose load, hypoglycaemia and exercise, there is a lot of evidence that peripheral loci are important as well in the initiation of a response to a potential threat to glucose homeostasis. However, little information exists about the relative involvement of these peripheral primary afferents in glucose homeostatic regulatory mechanisms, despite the fact that the entire gastro-intestinal tract –including important glucose sensing organs such as liver and pancreas- are innervated by vagal afferents (2).

The importance of sensory (afferent) nerves, as Aδ and C-fibers, in the control of glucose homeostasis has been shown in a number of studies. Although their exact function is not yet established, it appears that after treatment with the neurotoxin capsaicin, which destroys small – and unmyelinated fibers (11), animals show an improved glucose tolerance accompanied with an increased early insulin response after an intravenous glucose injection (15). This improved glucose tolerance after capsaicin treatment was also seen during an oral glucose tolerance test, although the insulin response was not affected (8). These results could indicate that capsaicin sensitive sensory nerves are involved in the regulation of insulin sensitivity. Supporting evidence was obtained by Zhou et al. (28) and Koopmans et al. (17), who found strong indications that neonatal capsaicin treatment leads to an improved insulin sensitivity. However, so far the mechanism responsible for these effects remains unclear. Calcitonin-gene related peptide (CGRP), the main neuropeptide present in capsaicin sensitive nerves, is shown to affect islet hormone secretion by antagonizing the effect of insulin in vitro (22) as well as in vivo (6, 19, 23), which might be an explanation for the observed effects on insulin sensitivity after capsaicin treatment. Modulation by sensory nerves can be due to a local effector loop, but can also be mediated via afferent vagal glucose sensors present in the portal liver system. These are responsible for reflex modulation of the efferent pancreatic vagus nerve activity that can be abolished by hepatic vagotomy (21).

Furthermore, the improved glucose tolerance after capsaicin deafferentation might be mediated by glucagon. Insulin and glucagon are considered as the key regulatory hormones for glucose homeostasis (14). It appears that the absolute levels of glucagon or the ratios of glucagon to insulin are often elevated in various forms of diabetes in both animal and human subjects (3, 27). This indicates that glucagon plays a major role in the regulation of glucose homeostasis. As a consequence of the lack of afferent input, capsaicin-treated animals might have a modified efferent output and therefore a different basal glucagon secretion in comparison with vehicle-treated animals.

Despite the evidence that capsaicin-sensitive sensory nerve fibres are involved in the regulation of insulin sensitivity, neither the importance in the

detection of glucose nor the underlying mechanisms are fully understood. Therefore, as part of a series experiments aimed at this problem, the present study is performed to investigate whether afferent nerves are involved in the detection for glucose. We chose to do this by intravenous infusion instead of an oral glucose tolerance test since an oral glucose tolerance test has also the confounding factors of receptors in the oral cavity that possibly play a role in the first phase of insulin release (25). Three different glucose concentrations were used and infused over a period of 30 minutes in capsaicin-treated animals and their vehicle treated controls. Previous studies on intravenous glucose tolerance tests in capsaicin-treated animals used a single glucose injection. The latter causes a short and acute disturbance in glucose homeostasis. In contrast, a glucose load over a longer period requires a continuous fine tuned physiological response to the glucose load. The pattern we normally observe during a glucose infusion is that animals have a stable, but higher level of blood glucose during the second insulin phase of the infusion period. In other words, the animals have reached a new homeostatic glucose set point.

The present set up, allows us to determine whether capsaicin-treated animals are able to respond in the same fine tuned manner as their controls. To this end, apart from glucose and insulin, glucagon levels were measured in order to determine whether plasma changes in this insulin antagonistic hormone might contribute to the observed effects.

2 MATERIAL AND METHODS

2.1 Animals and housing

Twenty Male Wistar rats, bred in our own laboratory were used and housed in climate-controlled rooms (22 °C ± 2) under a 12h: 12h light-dark cycle (lights on at 8:00 am). Food and water were ad libitum available, unless mentioned otherwise. Capsaicin-treated pups and vehicle-treated pups grew up separately -to avoid selective mother care- in litters of 5-9 pups, in the proportion 5-7 male, 2 female. After weaning, at the age of 23 days, rats were individually housed in clear plexiglass cages (25x25x30 cm).

2.2 Capsaicin treatment

Capsaicin (8-methyl-N-vallinyl 6 nonenamide, 50 mg/kg; Sigma Chemical, Netherlands) was given neonatally (n=10) by a subcutaneous (s.c.) injection. This was done under 100% O_2 conditions to avoid hypoxia. Capsaicin was dissolved in a vehicle consisting of 10% ethanol (10%) and 5% cremophore-0.9% sodium chloride solution (90%). As a control, vehicle solution was injected s.c. (n =10). Each animal was given the same volume of 50 µl based on an average weight of the pups of 8 grams.

At injection, both groups did not differ in body weight (capsaicin 7.9 g ± 0.09; control 7.9 g ± 0.09). After treatment, body weight was measured weekly, but no significant differences were observed between both groups throughout the experiment (see fig. 1). 1 capsaicin animal died during the nursing period and 2 animals died during the experimental period so we ended with a number of 5 to 7 animals per group.

An eye wipe response (0.1% capsaicin solution) was done at the age of 3 months in order to test the effectiveness of the capsaicin treatment. None of the capsaicin-treated animals did respond to the test and all animals were therefore included in the experiment.

2.3 Surgery

Double heart catheters were implanted bilaterally in the vena jugularis under general anaesthesia of isoflurane/N_2/O_2; fynadine (0.01 ml/ 100g body weight) was given s.c. as post surgical analgesia. Animals had at least 2 weeks of recovery before the start of experiments.

2.4 Experimental procedure and chemical determination

An intravenous glucose tolerance test was performed. Glucose was dissolved in 0.9 % sodium chloride (5 g, 10 g or 15 g in 100 ml). Each animal received 3 times an IVGTT concentration with at least 3 days in between. All treatments were randomly assigned. All experiments were performed in the light period between 12:00 am and 1:00 pm. Animals were shortly food deprived for 3 hours before the experiment started.

In order to allow stress free blood sampling, animals were connected to the tubing cannula at least half an hour before basal samples were taken.

Glucose was infused during 30 minutes at a rate of 5 mg, 10 mg or 15 mg per minute and a rat received consequently a glucose load of 150, 300 or 450 mg respectively. Samples of 0.2 ml were taken at –11, -1, 1, 3, 5, 10, 15, 20, 25, 30, 40 and 50 minutes and mixed with 5 µl of heparin to avoid blood clotting. Glucose, insulin and glucagon concentrations were measured and blood and plasma samples were stored at – 20 °C until analysis. Blood glucose levels were measured by the ferrocyanide method of Hoffman; plasma level of insulin, and glucagon were measured by commercial radioimmunoassay kits (Linco Research; RI-13K, GL-32K respectively).

2.5 Statistical analysis

The results are expressed as means ± SEM. Analysis of variance repeated measurements (ANOVA) followed by LSD test as post hoc tests were used for statistical evaluation. Testing for significant differences were performed from the start of glucose infusion till end of infusion (0-30 min). Student's t-test was used for unpaired observations. A P value of < 0.05 was considered significant. Area under the curve was measured from point zero (average of basal values) till 40 minutes after the start of the infusion.

3. RESULTS

Figure 1 shows that there are no significant differences in the growth curve between capsaicin-treated and vehicle-treated animals. Also at start of the experiment, there were no differences in body weight between capsaicin-treated and vehicle-treated animals (398 g ± 6.7 vs. 402 g ± 10.1, NS).

Figure 2 shows the changes in blood glucose levels (A), insulin (B) and glucagon (C) plasma over time during the 30 minute 150, 300 and 450 mg i.v. glucose infusion. ANOVA repeated measurements revealed that the 150, 300 and 450 mg glucose load induced significant changes of glucose ($F_{9, 108} = 27.82$; $F_{9, 90} = 92.63$; $F_{9, 81} = 85.27$ resp., $p < 0.001$) and insulin ($F_{8, 96} = 7.39$; $F_{5, 55} = 5.72$; $F_{8, 112} = 32.99$ resp., $p < 0.001$). Infusions of 150, 300 and 450 mg glucose did not induce significant changes in glucagon levels ($F_{9, 108} = 1.71$; $F_{9, 90} = 4.41$, $F_{9, 54} = 3.48$ resp., NS). For plasma glucose, there were no significant time ×

group interactions for all 3 glucose infusions ($F_{9, 108} = 0.58$; $F_{9, 90} = 0.51$; $F_{9, 81} = 0.47$ resp., NS). Also, there were no differences in glucose levels between capsaicin treated and vehicle control animals during 150, 300 and 450 mg glucose infusions ($F_{1, 12} = 0.60$; $F_{1, 10} = 2.39$; $F_{1, 9} = 1.53$ resp., NS). In contrast, plasma insulin levels did show significant effects of capsaicin treatment. Independently of the total glucose load (150, 300 and 450 mg), all capsaicin-treated rats had a significantly lower insulin response compared to their vehicle controls ($F_{1, 12} = 7.26$; $F_{1, 11} = 6.22$; $F_{1, 14} = 5.62$ resp., $p < 0.05$).

The early insulin response did not differ significantly between vehicle-treated and capsaicin-treated animals when the amount of 450 mg (6.52 ng/ml ± 1.22 vs. 5.92 ng/ml ± 0.63 resp., NS), 300 mg (5.08 ng/ml ± 0.32 vs. 5.93 ng/ml ± 1.03, NS) or 150 mg (4.25 ng/ml ± 2.29 vs. 3.35 ng/ml± 0.24 resp., NS) was infused. The differences between capsaicin and vehicle treated animals are particularly clear in the second phase of the insulin response. Also, there was no significant time × group interaction for insulin levels during the 150 and 300 mg glucose infusion ($F_{8,96} = 1.49$; $F_{5,55} = 1.71$ resp., NS). In contrast, the highest glucose load of 450 mg did result in significant time × group interaction for insulin plasma levels ($F_{1,112} = 2.79$, $p < 0.01$). As for glucagon plasma levels, capsaicin treated animals did not show different glucagon levels during the 150, 300 and 450 mg glucose infusion compared to vehicle controls ($F_{1, 12} = 0.65$; $F_{1, 6} = 3.24$; $F_{1, 10} = 1.91$ resp, NS).

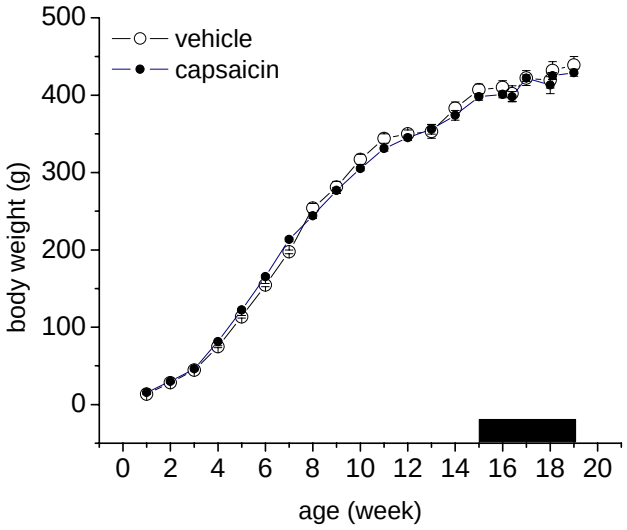

Figure 1
Growth curve of capsaicin-treated and vehicle-treated animals. Black bar represents the experimental period.

Table 1 shows the basal levels of glucose, insulin and glucagon. No significant differences were found between vehicle-treated and capsaicin-treated animals, except that basal glucose levels before 300 mg i.v. glucose infusion started was significantly lower in capsaicin-treated animals (5.1 mM ± 0.13 vs. 4.6 mM ± 0.09, $P<0.01$). However, since basal levels before the start of 150 mg or 450 mg i.v. glucose infusion did not differ between vehicle-treated and capsaicin-treated animals, it could be questioned what the significance is of this result.

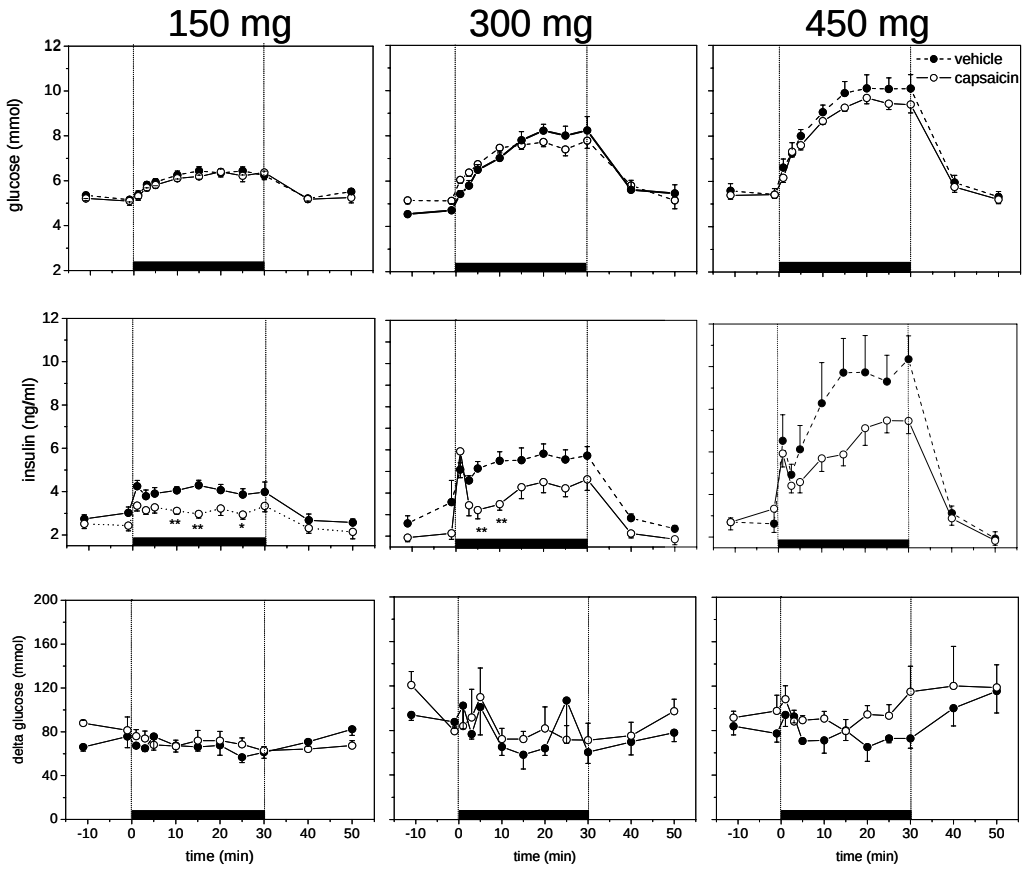

Figure 2

Changes in plasma levels of glucose (A), insulin (B) and glucagon (C) before, during and after i.v. infusion of 150, 300 and 450 mg of glucose in CAP and VEH rats. Insulin responses to an IVGTT are lower in CAP rats., ** $p < 0.05$ and $p < 0.01$ respectively.*

Table 1

Basal values of glucose, insulin and glucagon before the start of infusion of 150 mg (5 mg/ml), 300 mg (10 mg/ml) and 450 mg (15 mg/ml). Basal values are the average of basal measurements 11 minutes and 1 minute before start of the infusion.

Infusion	Glucose (mM)		Insulin (ng/ml)		Glucagon (pg/ml)	
	Vehicle	Capsaicin	Vehicle	Capsaicin	Vehicle	Capsaicin
5 mg/ml	5.3 ± 0.11	5.2 ± 0.14	2.9 ± 2.24	2.5 ± 0.22	75.4 ± 9.68	84.7 ± 6.84
10 mg/ml	5.1 ± 0.13	4.6 ± 0.09 **	2.7 ± 0.27	2.0 ± 0.21	90.8 ± 5.03	123.1 ± 10.76
15 mg/ml	5.5 ± 0.27	5.8 ± 0.15	2.7 ± 0.11	2.5 ± 0.36	80.7 ± 4.11	94.9 ± 7.3

Values are averages ± SEM

** Significant difference between capsaicin vs. vehicle-treated animals (2-tailed t-test; $P < 0.01$)

4 DISCUSSION

The main result of this study is that neonatal capsaicin-treated male Wistar rats show a diminished insulin response to an intravenous glucose infusion, while there is no difference in glucose tolerance when 150 mg, 300 or 450 mg glucose loads are infused. Glucagon levels do not change during 150 and 450 mg glucose infusion either in deafferentated rats or their controls. Yet, 300 mg glucose infusion resulted in a decrease in glucagon levels. Also, there are no significant differences between capsaicin- and vehicle-treated animals considering baseline plasma glucose and glucagon levels. This shows that a fine-tuned response to a glucose infusion does not depend (solely) on capsaicin-sensitive sensory nerves, since capsaicin-treated animals are normoglycemic during an intravenous glucose infusion.

Our results are partly in accordance with Karlsson et al. (15) and Guillot et al. (8), who found an improvement of glucose tolerance in capsaicin-treated animals after a glucose challenge that was accompanied by an enhancement of the early insulin response, followed by lower insulin levels in capsaicin-treated animals (15). Another study found that insulin secretion was the same as controls (8). Yet in our study, capsaicin-treated animals exhibit a reduced insulin response and there are no significant differences concerning the early insulin response. This discrepancy between the various studies may be due to the fact that Karlsson et al. (15) gave only one injection, while in our experiments the glucose infusion persisted for 30 minutes, which may partly have blunted the effect of this increased early insulin response. Furthermore, they used mice in their experiment which also might account for the observed differences with our results.

Since the lower insulin response is accompanied with normal glucose tolerance in capsaicin- treated animals, results of the present experiments suggest that insulin sensitivity is increased. Earlier data of Zhou et al. (28) and Koopmans et al. (17) indicated that after capsaicin treatment insulin sensitivity indeed improves. Although with the present set up of our experiment it remains unclear if the observed effects are due solely to increased insulin sensitivity, we do demonstrate that capsaicin-sensitive nerves are involved in insulin secretion. Another explanation could be that

capsaicin-treated animals have an increased non-insulin-dependent glucose uptake and, as a result, a smaller insulin response might be required to normalize blood glucose levels.

It is unlikely that factors that modulate insulin sensitivity like body weight and nutritional state could explain our results. It is known that a lower body weight can contribute to increased insulin sensitivity and the period of fasting can influence the response of insulin (26). In our experiments, capsaicin treatment did not result in a different body weight compared to vehicle treatment. No major differences concerning nutritional state are expected, since all animals were shortly food-deprived for 3 hours in the light period before the experiment started. We do not know if meal patterns differed between capsaicin-treated and vehicle-treated animals, which might result in different fasting periods before the experiment. However, such an explanation seems unlikely because this is usually associated with increased glucose levels (26), which is not the case in the present experiments.

The reduced insulin response might be a result of modifications of the responsiveness of the B-cells in capsaicin-treated animals. In vitro experiments on isolated islets exposed to capsaicin for several days showed no effect on B- cell function. Karlsson et al. (15) and Koopmans et al. (17) do not find altered islet secretory capacity in capsaicin-treated animals either. However, changes on islet level are reported since systemic capsaicin treatment led to an increase in both whole pancreatic and islet blood, whereas fractional islet blood flow was decreased when compared with vehicle-treated rats (5). This may result in a different insulin output as well.

Altered functioning of the catecholamine system could also contribute to the differences in glucohomeostatic mechanisms in capsaicin-treated animals in comparison with controls. Catecholamines limit glucose utilization, increase glucose production from the liver, inhibit insulin secretion and stimulate glucagon secretion (7). However, contradictory results are reported. Zhou et al.(28) found increased catecholamine levels as a response to insulin induced hypoglycaemia after neonatal capsaicin treatment, whereas Karlsson et al. (15) did not find a significant effect on catecholamine levels. Although we cannot exclude the contribution of altered catecholamine plasma levels to the observed effects on insulin secretion, this explanation seems unlikely in view of

the fact that no major changes in catecholamine levels are generally observed during an intravenous glucose tolerance test.

Glucagon is another insulin antagonistic hormone that might contribute to changes in plasma glucose levels. However, no major differences were found in plasma glucagon levels and could therefore not explain the present results. This does not confirm the data of Karlsson et al. (15), who observed an increased plasma glucagon level 10 minutes after one single glucose injection in capsaicin-treated animals. In addition, Karlsson et al. (16) found a reduced glucagon response to a 2-deoxy-D-glucose injection in capsaicin-treated rats, while insulin response was not affected. He concluded that capsaicin-sensitive nerves are involved in the regulation of glucagon secretion. The present results show that glucose homeostatic regulation via afferent nerves can also be modulated without glucagon as mediator.

Niijima (20) has shown that peripheral glucose-sensitive receptors exist in the liver. There are many data pointing clearly at the importance of vagal afferents for the detection of glucose and for the glucohomeostatic control (9, 10, 15, 17, 21, 28). However, conflicting data exist as well. Jackson et al. (12, 13) and Cardin et al. (4) raised some questions about the importance of vagal afferents for hypoglycemic detection, because they found that a functioning vagus nerve was not necessary for a complete counterregulatory hormone response to moderate hypoglycaemia. Since substantial afferent innervation of the portohepatis ascends via the sympathetic nervous system, it could be that hepatic glucosensors communicate with the brain via these afferents (12). However, it is as yet unclear whether the detection of hypoglycemic conditions is principally different from hyperglycemic conditions. Our results suggest that detection of hyperglycaemia does not solely depend on capsaicin sensitive nerves, since an infusion of different amounts of glucose results in a normoglycemic response accompanied with a fine tuned insulin response. Our data do show that capsaicin sensitive afferents are involved in insulin secretion to a certain glucose load. The modified insulin response in capsaicin-treated animals might reflect a change in efferent signalling as a result of the lack of afferent signalling; or as suggested by Karlsson et al. (15), it might be that capsaicin-sensitive nerves display their effects locally by intrinsic nerves of the pancreas or by an afferent limb of a neural reflex regulation of glucose

homeostasis. Since there are strong indications that insulin sensitivity has changed after capsaicin treatment, the hypothesis arises that capsaicin-sensitive sensory afferents might be important in the (down) regulation of insulin sensitivity.

In summary, these results suggest that capsaicin-sensitive afferents are not primarily involved in glucose detection. We showed that neonatal capsaicin-treatment in male Wistar rats leads to a diminished insulin response to an intravenous glucose infusion. It appears that capsaicin-sensitive sensory nerve fibres are important in the regulation of glucose-stimulated insulin secretion and/or in the regulation of insulin sensitivity. Glucagon levels do not differ between capsaicin-treated and vehicle-treated animals and could therefore not explain the observed effects. It is not excluded that present observations are due to changes on a secondary level after neonatal capsaicin treatment such as non-insulin-dependent glucose uptake, modified efferent output and/or local blood flow modifications on islet level. Since modification on insulin secretion ((15); this study) as well as on glucagon secretion (16) are reported, it is tempting to hypothesize that capsaicin-sensitive sensory nerves might play a role in the onset and development of pathologies where glucohomeostatic mechanisms are disturbed as is in Diabetes II.

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