Originals

Assessment of an Algorithm for the Artificial B-Cell Using the Normal Insulin-Glucose Relationship in Diabetic Dogs and Men

U. Fischer, E. Jutzi, H. Bombor, E.-J. Freyse, E. Salzsieder, G. Albrecht, W. Besch, and W. Bruns

Central Institute for Diabetes "Gerhardt Katsch", Karlsburg, GDR

Summary. Insulin secretion rates after glucose loading were calculated from peripheral venous IRI concentrations considering half life and distribution space of exogenous insulin in normal men and dogs. The coefficients of multiple linear regression analysis between insulin secretion rates and plasma glucose (level and order and rate of change) were used as algorithm parameters in glucose-controlled insulin infusions. These were carried out in each dog based on individual estimations before the induction of diabetes but in the diabetic patients based on values derived from a group of normal subjects. Using this formula, nearly normal patterns of glucose and of insulin were observed in diabetic men and dogs under basal conditions and after IV glucose loading but not after meals. This algorithm enables selection of the parameters prospectively. The effect of a parameter combination depends on insulin sensitivity and it should be appropriately adapted. In the diabetic patients there was no predictable influence of the brittle or stable characteristics of the disease nor of insulin antibodies on the glucose curves obtained with glucose controlled insulin infusions.

Key words: Artificial B-cell, algorithm, glucose-insulin relationship, regression analysis, insulin secretion, insulin half-life, human diabetes mellitus, experimental diabetes mellitus.

At present optimal control of blood glucose concentration in diabetes can be achieved by a continuous intravenous glucose-controlled insulin infusion (GCII)[1] [21, 32]. The infusion systems used (GCIIS)

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[1] Abbreviations: GCII – glucose-controlled insulin infusion; GCIIS – glucose-controlled insulin infusion system; IRI – immunoreactive insulin; OGTT – oral glucose tolerance test; PG – plasma glucose

apply the tanh function [3, 4, 22, 36] or other non-linear [6, 7] or linear [21, 23] algorithms to delineate the glucose-insulin relationship. Improvement of some of these algorithms has been achieved by so-called dynamic or derivative control [7, 21]. Appropriate algorithm parameters can, however, only be selected empirically. Thus we earlier derived a formula for the artificial B-cell based on a multiple regression analysis between plasma glucose and insulin secretion rates in response to a glucose stimulus in normal subjects [11]. The resulting regression coefficients provided the algorithm parameters for GCII in diabetes. Principally, this method allows the prospective and individual evaluation of the constants.

This paper deals with the further experimental investigation in men and dogs and preliminary clinical application of a GCIIS based on such algorithms.

Materials and Methods

The groups of experimental animals and men used in the experiments are show in Table 1 as are the investigations performed. One group of dogs of both sexes was investigated before and after the induction of diabetes, another group was observed without diabetes only; three different groups of normal men were tested, and one group of diabetic patients (Table 2) was investigated.

Animal Experiments

Two to four weeks before the induction of diabetes the dogs (normal group (1)) had been submitted to at least one glucose test (OGTT 1.0 g/kg or non-primed IV glucose infusion with 12 or 16 mg/kg/min for 60 min) and to an estimation of insulin half-life and distribution space by IV injection of 50 mU/kg Actrapid insulin. Details of these procedures have been described previously [11]. The animals were made diabetic by partial pancreatectomy and intraoperative administration of 2 mg/kg streptozotocin (Upjohn, Kalamazoo, lot No. BV 71-221) into the pancreaticoduodenal artery. If necessary another streptozotocin dose (15 mg/kg) was given intravenously three to six days after the operation.

The diabetes was treated with three daily injections of soluble pork insulin (Actrapid-MC). The animals received two or three

Table 1. Experimental groups

		n	Body weight (kg)	Age	Duration of diabetes	Type[d] of investigation	Purpose of the investigation
Dogs	Normal (1)	12	29 ± 1	28 ± 3 months	–	OGTT, IV glucose infusion, IV insulin injection, mixed meal	Algorithm parameters, insulin half life, comparison of the PG curves with those in diabetics on GCII
	The same animals made diabetic	12	29 ± 1	40 ± 3 months	10 ± 2[a] months	GCII with meals or glucose loads	Comparison with normals of diabetics treated with GCII programmed individually
	Normal (2)	10	31 ± 1	27 ± 4 months	–	Direct in vivo assessment of insulin secretion rate	Comparison with the insulin secretion rates as calculated by formula (1) from the peripheral venous IRI concentration
Men	Normal (1)	7	68 ± 3[b]	26 ± 1 years	–	OGTT	Algorithm parameters
	Normal (2)	8	59 ± 3[b]	22 ± 1 years	–	IV glucose infusion	Algorithm parameters
	Normal (3)	5	70 ± 4[b]	31 ± 4 years	–	Mixed meal	Comparison with GCII action in diabetics
	Diabetics[c]	12	67 ± 2[b]	32 ± 2 years	14 ± 2 years	IV insulin injection, GCII with mixed meals	Insulin half life, comparison with normals of diabetics treated with GCII using a standard algorithm

[a] Mean duration during the whole time of the study
[b] All values within the limits of 100 ± 3% (mean ± SEM) of the ideal weight
[c] For details see Table 2
[d] See Methods section for details

meals per day (depending on body weight) consisting of meat and oats and exercised for three h/day. The experimental observations in the diabetic state were performed in the conscious animals starting at 0700 h, 13 h after the last meal and the last SC injection of insulin. The animals had a chronically implanted jugular venous catheter for the insulin infusions. They were treated by GCII for 8 to 40 h as described below. During GCII the animals were given OGTT's, glucose infusion tests, or normal meals (1000 g meat). Not all animals were submitted to each test. At the time of study there were no circulating insulin antibodies, thus IRI represented the concentrations of free biologically active insulin, as in normal dogs.

In addition laparotomies were performed in non-diabetic dogs (normal group (2)) under general anaesthesia. Indwelling catheters for sampling of portal, intestinal, hepatic and peripheral venous and arterial blood for IRI analyses were inserted as were non-cannulating flowprobes for the electromagnetic measurement of pancreatic and hepatic blood flows. From these measurements the rates of net pancreatic insulin secretion and the hepatic balances of insulin were estimated and they were compared with insulin secretion rate calculations as presented below. The details of this experimental procedure and of the calculations have been described previously [9, 12].

Human Studies

The three groups of normal male volunteers (Table 1) were investigated to obtain algorithm parameters and normal plasma glucose curves after a meal. They were medical students or members of the laboratory staff. GCII was realized in 12 male diabetic patients (Table 2) during the first week of their hospital stay which was routine for stabilisation of diabetes. No other drugs were taken by the patients and by the control persons. All healthy subjects and all patients taking part were informed according to the declaration of Helsinki (38) of the nature of the study and gave their consent to participate. Ketosis-prone patients with a relatively high need of insulin, and with difficult glycaemic control due to extreme intra- and inter-day variability of their plasma glucose levels in response to constant insulin therapy were defined as "brittle diabetics".

The GCII studies were performed at bed-rest and were started at 0400 h, 6 h after the last SC dose of soluble insulin. By varying this dose the plasma glucose level at the beginning of the experiment was intentionally varied. For getting blood specimens and for performing the infusions the patients had two indwelling plastic cannulas in peripheral veins and were treated by GCII for 5 to 11 h as described below. The initial glucose normalisation, the fasting steady state glucose level (any alterations smaller than ± 3 mg/dl) reached on GCII, and the response to one or two mixed standard meals of 500 Cal (60 g carbohydrates, 15 g fat, 27 g protein) were recorded. Normal subjects received similar mixed meals and also oral glucose tolerance tests.

Analyses, Algorithm and Glucose-controlled Insulin Infusion

In all experiments plasma glucose (Beckman-Analyzer®) was measured every 2.5 or 5 min. For the estimation of plasma IRI (40) the samples were incubated for 20 h in triplicate with antibody at 22° C and thereafter with tracer insulin for 90 min. Human insulin (WHO – standard, Code 66/304) was used as standard. The antibody-bound fraction was precipitated with ethanol in a final concentration of 77%. Day-to-day precision was 8.4% at

Table 2. General characteristics of the patients investigated. All males. The presence of insulin antibodies was demonstrated qualitatively in cases 3, 5 and 9

Patient	Age [years]	Duration of diabetes [years]	% ideal body weight	Daily SC insulin dose [IU]	Plasma glucose [mg/dl]		Brittle or stable	Serum creatinine [μmol/l]	Retino-pathy[b]
					Daily[a] mean	range			
1	42	19	96	38	181	(286–43)	brittle	84	I
2	27	13	94	26	248	(325–156)	stable	97	I
3	28	14	95	42	244	(426–109)	brittle	90	0
4	34	21	102	60	295	(494–156)	brittle	71	III
5	36	16	105	52	261	(359–120)	brittle	90	I
6	34	2	103	32	257	(325–146)	stable	77	0
7	28	17	126	58	240	(484–68)	brittle	104	I
8	34	13	103	38	342	(411–283)	brittle	83	I
9	22	2	94	32	207	(286–62)	stable	76	0
10	36	19	89	64	155	(361–55)	brittle	80	II
11	37	18	94	52	307	(463–198)	brittle	80	II
12	23	11	98	52	248	(390–99)	brittle	88	0

[a] 9 analyses within 24 hours

[b] 0 – no retinopathy; I to III, progressive severity of retinopathy

24.9 μU/ml, and 6.8% at 93.4 μU/ml. The lower detection limit was 3.2 μU/ml given as 2 SD of the zero control samples. Insulin antibodies were estimated by incubating plasma with ^{125}I-insulin at 22 °C for 2 h, using normal plasma as control. Free and antibody-bound iodoinsulin were separated by ethanol precipitation. If antibodies were present in the sample the ^{125}I-insulin binding was significantly ($p < 0.01$) higher than that of the control sera.

From a plot of log IRI against time after IV insulin injection, the distribution space of insulin and the half-life of exogenous insulin were obtained.

Using the IRI curves of normal subjects after glucose loading insulin secretion rates were calculated at each interval (for details [11]):

CISR	$= F \cdot IS [IRI_{t + \Delta t} - IRI_t (e^{-0.693 \cdot \Delta t/\tau})]$	(1)
CISR	= calculated insulin secretion rate [mU/Δt],	
F	= constant for the immediate hepatic insulin uptake from the total (portal + arterial) inflow (Table 3),	
IS	= distribution space of exogenous insulin [ml/kg],	
τ	= half life of exogenous insulin [min],	
IRI_t PG_t	= a value of IRI and of PG at the experimental time t,	
Δt	= measuring interval [min].	

Based on these data and on the simultaneous plasma glucose determinations three components of peripheral insulin supply were defined by multiple regression analysis:

|		
CISR	$= ID = a_o + a_1(PG_{t + \Delta t} - PG_b) + a_2(PG_{t + \Delta t} - PG_t)$	(2)
ID	= rate of insulin administration in GCII [mU/Δt],	
PG_b	= desired basal PG level [mg/dl],	
a_o	= basal secretion rate [mU/ Δt],	
a_1	= regression coefficient for insulin secretion depending proportionally on the glucose level [mU/Δt/ mg·dl^{-1}],	
a_2	= regression coefficient for insulin secretion depending both on the rate and on the order of the change of PG [mU/mg·dl^{-1}·Δt].	

For preparing the original data the early glucose-independent IRI increase during an OGTT [10] was not considered, and a sliding fit technique (moving average over three values) was used.

These three regression coefficients were used as indices for insulin dosage during GCII. They were taken individually in dogs and as the "mean normal human parameters" in the patients. Based on the coefficients, dosage matrices were obtained, consid-
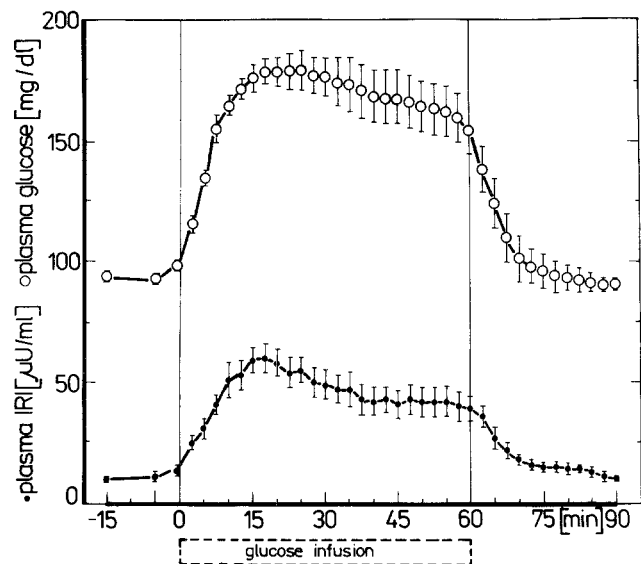

Fig. 1. Plasma glucose (O——O) and IRI (●——●) concentrations in 12 normal dogs before, during and after an IV glucose infusion of 60 min (16 mg/kg/min). Mean ± SEM

ering Δt, the body weight of the subject to be treated, and the concentration of the insulin solution (in most cases 120 mU/ml in 0.154 mol/l saline with 6% v/v mixed canine normal serum or with 4 g/l human albumin respectively).

Insulin doses were selected at a self-constructed programmer [34] controlling an external infusion pump (Infumat type 5157, MTA Kutesz, Hungary or Perfusor E, Braun-Melsungen, FRG). The infusion pattern consisted of alternating intervals with 2.5 min of a glucose-dependent dosage and 2.5 min of a glucose-independent basal dose (a_o). Analytical specimens for PG and IRI were obtained during the basal infusion periods. The whole delay between any glucose alteration and the resulting insulin dose was 2 min. The infusion was performed both in a rate-variable flow or in the form of constant-rate pulses of a variable duration. When appropriate means ± SEM values are given.
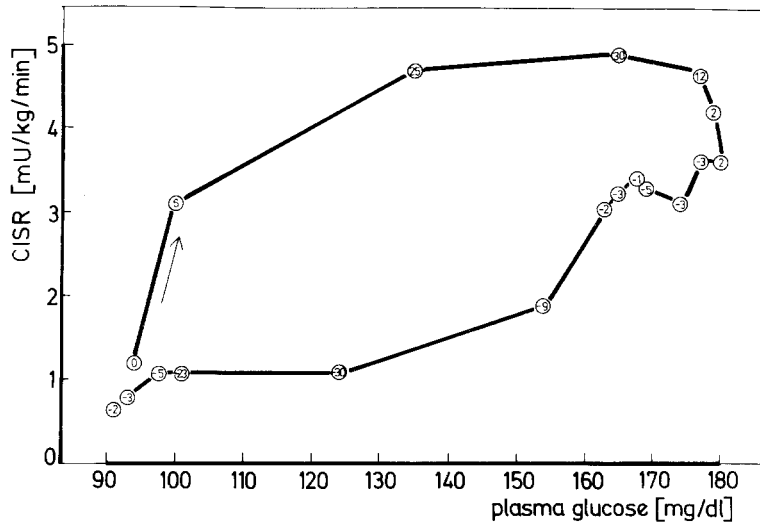
Fig. 2. Relation between the calculated insulin secretion rates (CISR) and the plasma glucose values with respect to the changes of plasma glucose during and after an IV glucose infusion in normal dogs. The values were taken from the mean curve presented in Figure 1 for intervals of 5 min beginning at -5 min. CISR was calculated according to formula (1) (see Methods)
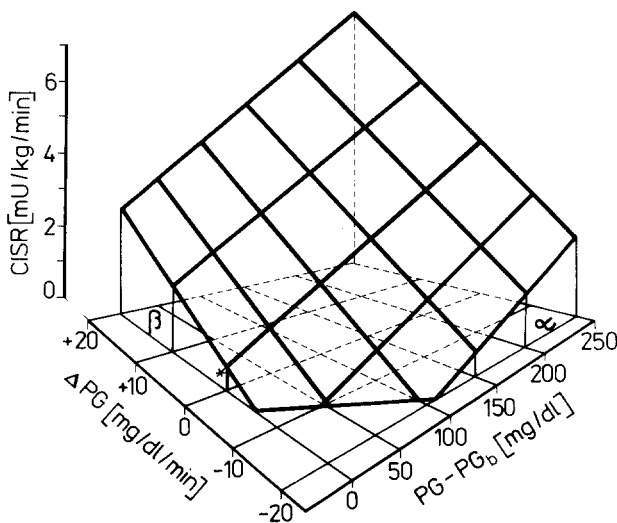
Fig. 3. Multiple regression analysis between the calculated insulin secretion rates (CISR), the difference of the actual to the basal PG value (PG-PG$_b$), and the rate of change of PG (Δ PG). The CISR and PG data were taken from the Figures 1 and 2 (IV glucose infusion in normal dogs, mean curve). Regression coefficients: tan α = a$_1$, tan β = a$_2$, the asterisk denotes the basal insulin secretion rate a$_0$. For comments and the numerical values see Table 4

Results

Experimental Proof of the Algorithm

In Figures 1–3 the insulin-glucose algorithm is graphically derived, based on data obtained from IV glucose infusions in normal dogs (Fig. 1). The apparent insulin secretion rates were calculated for each interval applying formula (1). These calculated insulin secretion rates depend not only on the actual plasma glucose level but also on the order and the rate of its change. This is demonstrated in Figure 2

using the mean curve of Figure 1. The numbers in the circles of Figure 2 denote the PG changes in the intervals; the coordinates of the circles are determined by a given insulin secretion rate and by the related PG level. It can be seen that at a given PG level the yield of insulin increases when the glucose concentration was increasing during the preceding interval. Thus multiple regression analysis was performed between the insulin secretion rates and the PG level and its rate of change. The difference between actual and basal PG was taken as a figure of the glucose level. The mean basal PG (Tables 4 and 6) concentration was defined as 90 mg/dl if no individual value differed by more than 8 mg/dl from this. In the three-dimensional graph (Fig. 3) the resulting regression function according to formula (2) is represented by a plane inclined by two angles. The tangent values of these angles give the regression coefficients a$_1$ and a$_2$ und the insulin secretion rate at the not changing basal PG is a$_0$. When in GCII (PG-PG$_b$) is < 0 the coefficient a$_1$ reduces the insulin dose to be infused as does a$_2$ when (PG$_{t+\Delta t}$–PG$_t$) is < 0. The upper limit for the validity of this algorithm was fixed at PG = 400 mg/dl. Higher values were assumed to be in the saturation range. During normal use however, such high values were never reached.

One crucial point in the parameter estimation is the assessment of the calculated insulin secretion rate. Pancreatic and hepatic blood flow recordings combined with pre- and postpancreatic and pre- and posthepatic IRI analyses provide the possibility to control these estimates directly. Table 3 demonstrates that 50% of hepatic (arterial + portal) insulin inflow were taken up in the liver of non-diabetic anaesthetised dogs both during normoglycaemia/normoinsulinaemia and during steady state hyperglycaemia/hyperinsulinaemia. This was considered in

Table 3. Insulin secretion rates and hepatic insulin balances in non-diabetic, anaesthetised, laparotomised dogs. Insulin secretion rates were estimated as the product of pancreatic blood flow and of arteriovenous IRI concentration difference; hepatic insulin inflow was estimated as the sum of portal and arterial inflows (each variable was the product of concentration and of the respective blood flow); hepatic insulin outflow was the product of hepatic venous IRI and of the portal + hepatic arterial blood flows. All blood flow monitorings were done continuously by a non-cannulating electromagnetic flowprobe. Each individual was considered with the mean of 3 estimates in intervals of 5 min performed before or at the end of an IV glucose infusion (30 mg/kg/min × 30 min). The data represent the mean ± SEM of 10 animals (weight 31 ± 1 kg)

	Fasting	Glucose infusion	Significance
Arterial plasma glucose [mg/dl]	116 ±7	412 ±16	p < 0.01
Arterial plasma IRI [μU/ml]	29 ±4	143 ± 8	p < 0.01
Pancreatic insulin secretion [mU/min]	5.6±1.6	30.0± 6.2	p < 0.01
Hepatic insulin inflow [mU/min]	17.6±3.0	86.3±11.7	p < 0.01
Hepatic insulin outflow [mU/min]	8.0±1.9	42.0± 6.3	p < 0.01
Hepatic insulin uptake [%]	51.5±6.2	49.0± 4.1	

insulin secretion rate [mU/Δt] as estimated from pancreatic blood flow and arterio-portal IRI difference

Fig. 4. Relation between directly estimated insulin secretion rates (x axis) and those calculated from the peripheral venous IRI concentrations according to formula (1) (y axis) in an anaesthetised laparotomised dog bearing multiple catheters and electromagnetic blood flowprobes. A primed IV glucose infusion was given for 120 min (priming dose 250 mg/kg, infusion 15 mg/kg/min), Δt is between 1 and 10 min from 0 to 180 min. Body weight 22.6 kg, insulin half life 4.0 min, insulin space 160 ml/kg. The regression is $y = 20.3 + 0.99 \, x \, (r = 0.90)$

the calculation of insulin secretion rates from peripheral venous IRI concentrations by the factor F = 2 in formula (1).
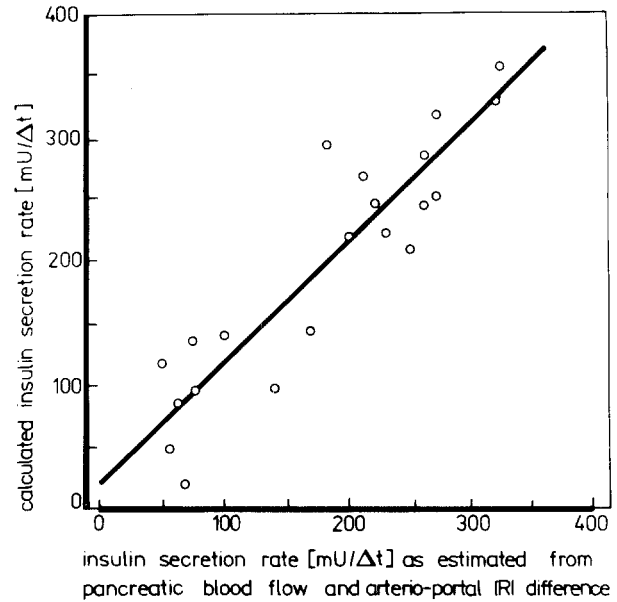
In Figure 4 directly estimated insulin secretion rates are compared with the calculated secreted rates, there was good agreement. Additionally the measurement of IRI concentration during a GCII provides the possibility to compare the real insulin dosage (ID) per interval (Δt) with that calculated from peripheral venous IRI concentration (apparent CISR) in diabetic dogs with no insulin antibodies. Theoretically both these figures should give identical data. From the experiments demonstrated in Figure 5 the following correlation was estimated:

$$ID = 0.85 \cdot CISR - 1.0 \, (n = 112, r = 0.89) \, [mU/ 5 \, min.].$$

Thus the posthepatic rate of insulin supply can be estimated accurately from peripheral venous IRI.

Individual Algorithm Parameters in Dogs

Table 4 shows the algorithm parameters of normal dogs. There were no significant differences between the estimates from OGTT and from IV glucose infusions respectively. The values, however, showed considerable intraindividual variation when the animals

were investigated at weekly intervals. This was applicable to both the basal and the glucose-dependent insulin yield. The interindividual parameter range was within the same range. The coefficients of variation were 64% (a_0), 45% (a_1), and 52% (a_2) respectively.
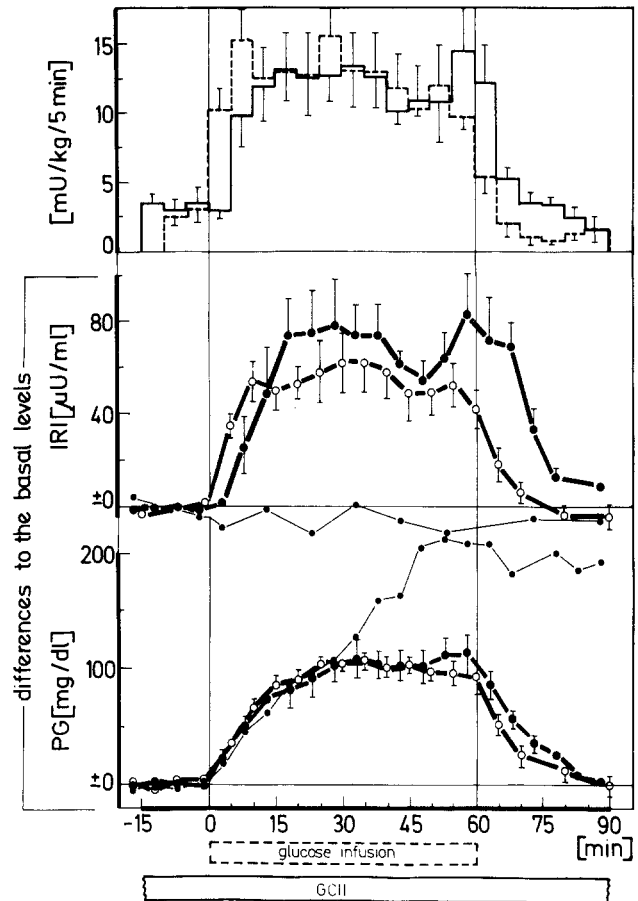
The insulin-glucose regression coefficients estimated in this way served as algorithm parameters in GCII after induction of diabetes. In dogs only the individually estimated parameters were used. Most animals exhibited the same PG curves and nearly the same IRI curves as in the non-diabetic state when they were submitted to a non-primed glucose infusion four to five hours after the beginning of a GCII (Fig. 5). The total insulin dose infused did not differ from that amount evaluated from the calculated insulin secretion rate (formula (1)) before induction of diabetes in the same animals. As was expected from the delay in glucose measurement and insulin administration both the dosage pattern and the IRI curve were somewhat delayed in relation to the normal curves.

Some of the diabetic dogs did not react with normal glucose curves to the individually estimated algorithm parameters. One example is presented in Table 5. In this case the animal's own formula as evaluated from test 1 did not allow reproduction of a

Table 4. Half life [min] and distribution space [ml/kg] of exogenous insulin, basal plasma glucose concentration [mg/dl] and the parameters of the multiple regression analysis between calculated insulin secretion rates [formula (1)], and the level (difference to the basal concentration) and the rate of change of plasma glucose in normal dogs. The regression parameters were evaluated from the plasma glucose and IRI curves of OGTT's or of IV glucose infusion tests. One individual dog is presented which was repeatedly investigated at weekly intervals; the data of all dogs are presented both as the mean ± SEM of the individual parameters or as the regression parameters of the mean curves. Mathematical analysis was performed as given in formula (2), and it is proved by the multiple correlation coefficients R (n = 72 pairs of data in OGTT and 36 in glucose infusion). a_0 [mU/kg/min] denotes the insulin secretion rate at the basal PG, a_1 [mU/kg/min/mg · dl^{-1}] is the coefficient for the glucose-proportional part of insulin secretion; a_2 [mU/kg/mg · dl^{-1}] relates to the PG change per minute

Subjects	Insulin distribution Half life [min]	Space [ml/kg]	OGTT No.	Basal PG [mg/dl]	a_0^*	a_1^*	a_2^*	R	IV glucose infusion No.	Basal PG [mg/dl]	a_0^*	a_1^*	a_2^*	R
Selected individual dog	4.5	119	1	94	0.59	0.0136	0.215	0.65	7	89	0.64	0.0117	0.199	0.93
			2	93	0.20	0.0181	0.145	0.88	8	84	1.00	0.0052	0.096	0.66
			3	83	0.66	0.0128	0.358	0.84	9	93	0.54	0.0142	0.126	0.77
			4	92	0.65	0.0047	0.184	0.67	10	91	0.63	0.0190	0.118	0.93
			5	81	0.96	0.0116	0.130	0.68	11	83	1.07	0.0234	0.163	0.89
			6	82	0.97	0.0147	0.185	0.55	12	85	0.94	0.0199	0.099	0.91
			Mean	88	0.67	0.0126	0.203	–	Mean	88	0.80	0.0156	0.134	–
			± SEM	2	0.12	0.0018	0.033		± SEM	2	0.09	0.0027	0.016	
All animals Mean (n = 12) ± SEM	3.1 0.2	115 6												
Regression analysis of the mean curves				96 2	0.49 0.09	0.0184 0.0024	0.119 0.018	0.96		92 2	0.66 0.07	0.0175 0.0034	0.093 0.019	0.96

* See legend for units

Fig. 5. Non-primed IV glucose infusion in 4 dogs before diabetes (O—O) and with GCII after induction of diabetes (●—●), one animal was additionally investigated after induction of diabetes but with the GCII interrupted when starting the glucose infusion (●—●). Results are shown as increments of the PG and IRI values from those found at the beginning of the test (mean ± SEM). The upper part shows the insulin secretion rates [calculated with formula (1)] before diabetes (— — —) and the insulin dosage pattern in GCII (——). Pretest values: PG 98 ± 2 mg/dl (controls), 107 ± 10 mg/dl (diabetics on GCII) and 95 mg/dl (diabetic with the GCII interrupted); IRI 15 ± 4 μU/ml, 20 ± 7 μU/ml, and 16 μU/ml respectively. The integrated insulin secretion rates from 0–90 min were 162 ± 21 mU/kg (calculated secretion in normals) and 163 ± 29 (GCII dose in diabetics). The algorithm parameters used in GCII were individually estimated before induction of diabetes

normal PG pattern during IV glucose infusion (test 2). These curves were, however, normalised when at the beginning of each GCII the individual formula was fitted to the actual individual insulin responsiveness (tests 6–9). In most cases very high doses of insulin were necessary, in relation to Table 4 it should also be stressed, that different formulae can produce very similar PG curves but that an extreme enhancement of basal secretion in relation to the factors a_1 and a_2 leads to a distinctly reduced basal PG

Table 5. Parameters of the plasma glucose and IRI curves, and need of insulin in a diabetic dog on glucose-controlled insulin infusions using different algorithm parameters. An additional IV glucose infusion of 60 min was given. The observations were done at weekly intervals (excepted that before diabetes). Experiments commenced at 0730 h (14 h after the last SC insulin injection), IV glucose was infused from 13.00 h. The algorithm parameters applied in test 2 were the animals own individual regression coefficients resulting from test 1 [according to the formulae (1) and (2)]. The parameters used in test 3 and 4 were arbitrarily selected; those of the test 5–9 resulted from the adaptation of the original formula to the actual insulin responsiveness. For this the initial PG fall (rapidity and the level reached) was taken as a measure. Insulin half life was 3.0 min before test 1, 2.6 min at the time of test 4, and 2.8 min after test 9. Insulin antibodies were not detected at any time

Expt. no.	Algorithm parameters[a]			Plasma glucose			Plasma IRI		
	a_0 [mU/kg/ min]	a_1 [mU/kg/ min/ $mg \cdot dl^{-1}$]	a_2 [mU/kg/ $mg \cdot dl^{-1}$]	Before GCII [mg/dl][b]	Before IV glucose [mg/dl][c]	Reactive area to IV glucose [mg/ $dl \cdot 90$ min]	Before GCII [μU/ml][a]	At the end of GCII [μU/ml][b]	Insulin dose on GCII [mU/kg/ 90 min]
1	Before diabetes, no GCII			—	98	5688	19	69	163[e]
2	0.76	0.015	0.156	411	100	8138	8	113	185
3	1.52	0.031	0.156	366	59	9335	3	174	343
4	0.38	0.008	0.078	416	121	11038	2	114	151
5	0.72	0.118	0.576	370	106	7400	10	211	739
6	0.75	0.123	0.600	462	110	6072	3	218	587
7	1.05	0.172	0.840	420	105	5350	12	268	746
8	0.98	0.160	0.780	430	98	5198	2	128	493
9	1.12	0.187	0.913	391	99	4760	1	341	779

[a] For explanation see Table 4
[b] The mean of two analyses within 15 min
[c] The mean of six analyses within 30 min
[d] The mean of four analyses within 10 min
[e] Calculated insulin secretion rate using formula (1)

level during GCII but does not improve glucose tolerance.

The influence of a GCII on the PG curve induced by a beef meal was investigated using the individual formulae (Fig. 6). In nondiabetic dogs there was no significant PG alteration. In diabetics under GCII, however, a small but significant and longlasting PG increase occurred. This was presumably due to insufficient insulin delivery (Fig. 6, upper part). The amount infused was considerably smaller than the calculated insulin secretion rate in nondiabetic animals under these conditions.

Application of the Present GCIIS in Diabetic Patients

For the GCII-treatment of human diabetes the algorithm parameters could only be obtained from studies in healthy subjects (Table 6). Basal insulin secretion (a_0) did not vary significantly between OGTT and glucose infusions, but the glucose dependent part of insulin supply (a_1, a_2) was higher when evaluated from the OGTT. Thus estimates from the mean normal OGTT curve were used.

In diabetic patients normoglycaemia was reached within 188 ± 19 min. This basal PG was 85 ± 3 mg/ dl (Table 7). No hypoglycaemia occurred and glucose
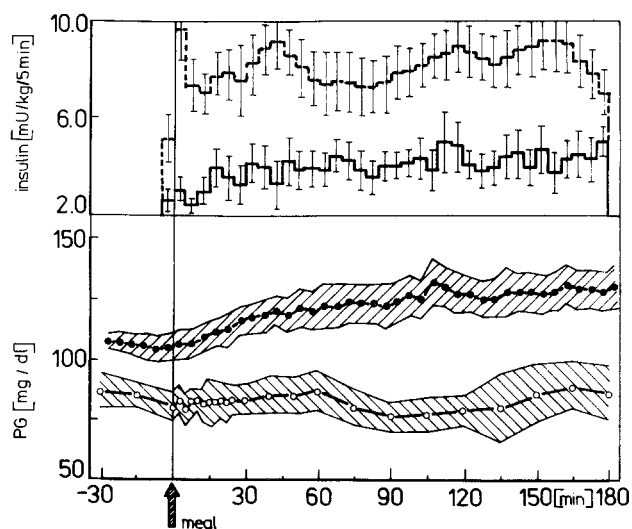
Fig. 6. Effect of a mixed meal on plasma glucose levels in normal dogs (O——O, n = 11) and in diabetic animals on GCII (●——●, n = 8). PG in the diabetic animals was normalised by GCII and maintained on a steady level for at least 30 min before the meal. The individually estimated algorithm parameters were used. The upper part shows the insulin secretion rates before diabetes (according to formula (1), — — —; from 0 to 180 min 298 ± 41 mU/kg were secreted) and the dosage pattern on GCII (———, from 0 to 180 min 143 ± 22 mU/kg were infused, p < 0.01 for the difference)

Table 6. Parameters of the multiple regression analysis between insulin secretion rates and the level and the rate of change of plasma glucose in normal men. Two groups of volunteers were investigated with an OGTT or an IV glucose infusion respectively as denoted in Table 1. See legend of Table 4 for further details. For the calculation of insulin secretion rate according to formula (1) the mean ± SEM of the insulin half life (3.6 ± 0.2 min) and of insulin space (148 ± 45 ml/kg) of the diabetic patients were taken

Group	Basal PG [mg/dl]	Test		Algorithm Parameters			
				a_0 [mU/kg/min]	a_1 [mU/kg/min/mg·dl]	a_2 [mU/kg/mg·dl]	R
I (n = 7)		OGTT	Mean of all	0.55	0.0145	0.192	–
			individuals ± SEM	0.09	0.0023	0.048	
	90		Mean curve[a]	0.54	0.0157	0.103	0.98
II (n = 8)	± 1	IV glucose	Mean of all	0.56	0.0089	0.046	–
		infusion	individuals ± SEM	0.08	0.0014	0.016	
			Mean curve	0.71	0.0077	0.100	0.94

[a] This parameter set was used in the experiments presented in Figure 7

Table 7. Plasma glucose characteristics and insulin requirement of the patients on GCII

Patient no.	Plasma glucose [mg/dl]				Duration of the meal-induced hyperglycaemia [min]	Insulin requirement		
	Level at the beginning[a]	Fall during the first hour	Steady state[b] before meal	Maximum after the meal[a]		for PG normalisation in the morning [mU/kg]	on PG steady state [mU/kg·min]	for compensation of the meal-induced hyperglycaemia [mU/kg]
1	229	25	89[c]	–	–	102	0.52	–
2	204	80	77	155	175	80	0.34	104
3	397	138	94[d]	168	175	226	–[d]	126
4	498	165	101	155	240	309	0.71	162
5	306	77	86	139	130	208	0.45	74
6	143	37	83	139	170	45	0.43	97
7	388	106	79[c]	–	–	287	0.37	–
8	439	106	75[c]	–	–	344	0.30	–
9	261	127	84	172	210	85	0.46	168
10	258	84	78	173	120	126	0.35	75
11	339	129	100	168	180	183	0.70	137
12	365	139	74[c, d]	–	–	185	–[d]	–

[a] The mean of 3 determinations within 15 min
[b] The mean of 6 determinations within 30 min
[c] No meal given
[d] There was still the tendency to decrease when the meal was offered or the study interrupted respectively

was not infused. During this period immediately before the meals the mean insulin requirement was 1.23 ± 0.10 mU/kg · min. Insulin dosage and plasma glucose pattern were independent of the patients characteristics (Table 2) and of the pretest PG concentration.

When the patients were normoglycaemic they were given a mixed meal (Fig. 7). The resulting glucose profile showed a higher peak and delayed normalisation in comparison with those of normal subjects. The maximum PG value was the same in stable and in brittle diabetics (155 ± 5 and 161 ± 7 mg/dl); "basal" levels were again reached after 169 ± 15 min

in both groups. In stable patients 6.8 ± 1.1 IU and in brittle patients 7.4 ± 0.8 IU insulin were given by GCII over the period of the meal. This was also less insulin than the calculated secretion rate in normal subjects. The three patients with insulin antibodies (Table 2) did not differ from the other diabetics in any GCII characteristics.

Discussion

The parameters of the present algorithm can be determined prospectively and individually. Its use in GCII provides the possibility to reach and to main-

tain normal basal and post-glucose profiles of PG and of IRI in the majority of diabetic subjects and to provide acceptable meal-time profiles. Long-term metabolic amelioration has also been attempted using this formula [11]; glucose administration to prevent hypoglycaemia was not necessary. In most cases normal blood concentrations of insulin could be kept.

Several algorithms have been used so far in artificial B-cells using electronic [7] or electromechanical [27, 28] equipment. The simplest tool is an on/off regulation as used earlier by Mirouze et al. [27]. Since this type clearly does not mimic the normal physiological response of B-cells it did not produce normal glucose profiles but considerable fluctuations.

Normally, glucose-stimulated insulin secretion in vitro exhibits saturation kinetics with a threshold phenomenon [25]. It also depends on the rate of change of glucose concentration [29]. A qualitatively similar pattern was demonstrated for the relationship of insulin concentration to the glucose level and to its rate of change in vivo [13]. Accordingly, Albisser et al. [3, 4] inaugurated the tanh function and Pfeiffer et al. [21, 32] used a fourth order parabola as an algorithm for the artificial B-cell. The parameters, however, can only be selected by experience and be optimised, if necessary, at random [5]. This uncertainty is more or less compensated by the different kinds of a dynamic or prospective consideration of any glucose alteration [3, 6, 7, 21 22]. Up to now, when applying these formulae it has often been necessary to infuse additional glucose in order to prevent hypoglycaemic episodes [24, 36].

The present algorithm was derived from the system of differential equations presented by Ackerman et al. [1]. After changing from IRI into the calculated insulin secretion rate, one of these equations was rearranged. A similar tool used by Kawamori et al. [20] is based on the regression analysis of the concentrations of glucose and of insulin and works with multiples of an independently assessed basal dose as an administration schedule. The metabolism of insulin and its action on glucose utilisation were also considered by Kruse-Jarres et al. [23] in simulating an extracorporal blood glucose regulating system. The basis of the algorithm described here is given by the calculation of insulin secretion rates. These figures do not correspond in all cases to those which are to be expected theoretically (see Fig. 4). The maximum difference was about ± 20%. This is probably due both to the analytical error and to the variation of actual hepatic handling of insulin (F).

An artificial B-cell cannot completely replace the well-timed aminogenic and enterohormonally-mediated insulin secretion after a mixed meal or after
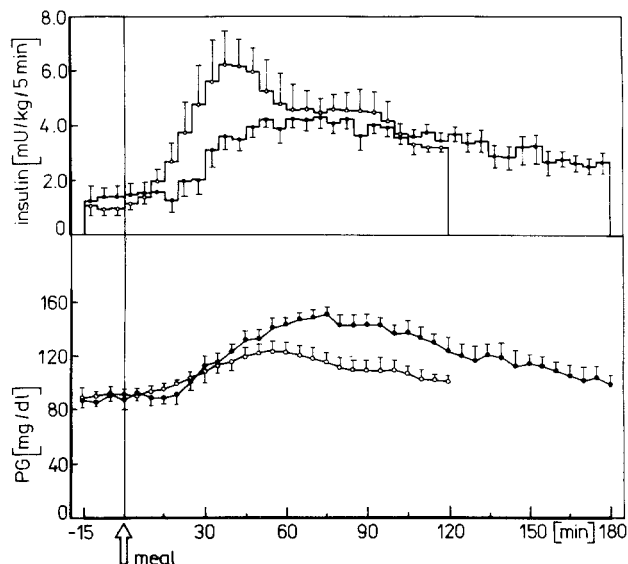
Fig. 7. Effect of a mixed meal on plasma glucose levels in normal men (O——O, n = 5) and in diabetic patients on GCII (●——●, n = 8). PG in the diabetics was normalised before the meal by GCII. The algorithm parameters of a "general man" (resulting from normal OGTT, see Table 6) were used. The upper part shows the insulin secretion rates [according to formula (1)] before diabetes and the insulin dosage pattern on GCII

intake of the typical canine food consisting mainly of proteins (Fig. 6 and 7). This is probably partly compensated by the so-called dynamic control parameters of the algorithm [21, 32]. Accordingly the importance of appropriate parameters for an early immediate reaction of insulin dosage has already been shown by Albisser et al. [4] in all situations with an increasing glycaemia. In the present system this function is fulfilled by a_2. Its linear consideration does not delay the normalisation of hyperglycaemic values and it does not produce inappropriate oscillations under basal conditions. However, better timing of the measuring and dosage intervals should further improve the results.

One of the basic problems in estimating the algorithm parameters is that of individualisation [26]. GCII does not primarily need individual formulae as has already been suggested by Horwitz et al. [18]. From the clinical viewpoint especially the apparently glucose-independent need of insulin a_0 must be taken into consideration [19]. It represents more or less the basal insulin requirement which is obviously connected with the actual insulin responsiveness [15]. The latter can vary considerably from day to day [11]. This basal rate (calculated as a_0 or really applied in GCII) is higher than often assumed (see Tables 6 and 7). Similar observations were obtained using the Biostator®. This is based on the fact that normally

hyperglycaemia-independent factors stimulate additionally insulin secretion and that in GCII insulin dosage is required by all minor glycaemic alterations.

In this study there was a great variability both of a_0 and of glucose-dependent insulin supply (a_1, a_2). The glycaemic reaction in GCII depended clearly also on the actual parameter value chosen and some diabetic would not manage with the algorithm constants as primarily estimated (see Table 5). Plasma insulin levels have always been proportionally related to insulin dosage (Table 5). Additionally in the literature no clear differences in insulin metabolism between normal and insulin-dependent diabetic subjects have been reported [30, 33]. Thus the different GCII effects are probably due to varying insulin sensitivity and the parameters should be adapted during the initial phase of a GCII. One approach to such adaptation is presented in Table 5 (tests 5–9) where the ratio of the mean insulin dose in PG steady state with the original formula to the theoretical basal insulin dose was used as a reinforcing factor during the further GCII run.

According to our experience in the diabetic patients a similar therapeutic rule is feasible starting with the mean parameters of a "normal man". More information is, however, needed on the relation of the metabolic effect of a parameter to the patient's data (e. g. degree of obesity, acute or chronic stress reactions, quality of preceding metabolic control). According to these preliminary results the applicability of a GCIIS does not strongly depend on the existence of a brittle or a more stable metabolic type or on the occurrence of insulin antibodies.

Our previous observations in diabetic dogs on GCII of a distinct and reproducible circadian pattern of plasma glucose [8, 11] points additionally to the necessity to adapt the algorithm parameters. There were similar findings in man. These results correspond well to the attachment of the glucoregulatory system to the circadian phase pattern of man [39]. It could be expected that time-dependent algorithms determined from such observations could provide the function principle for a more simple version of automated insulin therapy like the pre-programmed open-loop system [2, 14, 18, 31].

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Dr. U. Fischer
Central Institute for Diabetes
"Gerhard Katsch"
DDR-2201 Karlsburg
German Democratic Republic