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Review

Measurement of Insulin Absorption and Insulin Action

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ABSTRACT

For the practical implementation of every type of insulin therapy it is necessary to know both the time course of action of therapeutically used short- and long-acting insulin preparations and the factors influencing such time–action profiles. The only reliable way to obtain the required quantitative information about the pharmacokinetic and glucodynamic properties of insulin preparations has been the use of the euglycemic glucose clamp technique. The first studies with each new insulin formulation or insulin application technique should be performed with healthy subjects in order to have the most comparable study conditions. Thereafter, results from such clinical-experimental studies should be verified in similar studies with patients with diabetes. Earlier investigational approaches, which either had been limited to the determination of the pharmacokinetic properties of insulin preparations or had used the quantitative decrease of the blood glucose level as a measure of the pharmacodynamic properties, do not provide valid quantitative results. The proposed glucose clamp technique makes possible the quantitative study of the pharmacokinetic and pharmacodynamic properties of insulin preparations under comparative and reproducible conditions.

INTRODUCTION

THE AIM OF THIS REVIEW is to establish a uniform procedure to investigate the pharmacological properties of insulin preparations and alternative insulin administration forms. Up to now a number of different techniques have been and are still used to study the pharmacokinetic and pharmacodynamic properties of insulin. Even when it is stated that the same technique is being used, there frequently are differences in the manner and exactness in which studies are performed (even small details are very important!). This makes it difficult or impossible to compare the results of different studies, even if they are performed with the same insulin preparation/insulin administration form. In this review we will critically summarize the knowledge about the different approaches used and the pros and cons of the technique suggested to be used in the future, namely, the euglycemic glucose clamp technique.

The success of an insulin replacement therapy in terms of improved metabolic control depends on successfully covering the body's insulin requirements by adequate insulin replacement so that the rise in blood glucose after meals is as low as possible and that the

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hepatic glucose production is adequately suppressed between meals. With the use of intensive insulin therapy, patients with insulin-dependent diabetes mellitus aim at adequately substituting the considerably varying insulin requirements over the day by using an appropriate combination of subcutaneous (s.c.) injection of short-acting insulin before meals and long-acting insulin in the morning and at bedtime.[1–3] Prerequisite for such an optimized insulin therapy is the information about the pharmacodynamic properties of the insulin preparations used. The disadvantages of animal and human insulin preparations, especially with regard to the time–action profile of the blood glucose-lowering effect, have been shown to hamper the implementation of intensive insulin therapy[4]; nevertheless, it had been proven to be possible. The potential for a more successful implementation of intensive insulin therapy resides with the more suitable time–action profiles of novel insulin preparations (analogues) or novel insulin administration forms, *e.g.*, inhalation of insulin.

BASIC CONSIDERATIONS

The measurement of the concentration of a drug and its metabolites in the body over time allows the description of its pharmacokinetic properties in quantitative terms. The time course is determined by the processes involved in the absorption of a drug, its distribution, its biotransformation, and, finally, its elimination. The evaluation of the biological effects of a drug, derived from the drug–receptor interactions, as well as from the subsequent biological processes, allows the quantification of its pharmacodynamic properties. The purpose of pharmacodynamic studies of various insulin preparations is to define the time–action profile of their blood glucose-lowering effect.

WHY IS IT NECESSARY TO STUDY THE PHARMACODYNAMIC PROPERTIES OF INSULIN?

The blood glucose-lowering effect, as one of the most important metabolic effects of insulin, correlates with the change of insulin concentration in the blood, but there is a delay between changes in insulin concentration in the blood and changes in blood glucose. This shift between the serum insulin concentration and the time–action profile (hysteresis) depends on, among other things, the absorption rate of insulin. Thus, the shift is most pronounced after an intravenous (i.v.) administration of insulin. Metabolic activity can still be measured even after the blood insulin concentration has returned to basal values.[5–9]

Investigation of only the pharmacokinetic properties of insulin preparations ignores this hysteresis, which is based on the series of events occurring between the increase of the intravascular insulin concentration and the resulting metabolic activity. The metabolic activity (= increase of glucose utilization) of insulin administered by s.c. injection depends on:

- the fraction of the dose being absorbed
- the time course of absorption
- the fraction passing the endothelium of the capillaries and being transported into the interstitial space (transcapillary insulin transport, see below)
- the time the insulin requires to reach the cell surface
- the binding of insulin to the insulin receptors
- the activation of tyrosine kinase at the intracellular receptor part
- the subsequent translocation of glucose transporters from the inner cell to the cell membrane (and/or transporter activation)
- the current glucose transport
- the glucose phosphorylation
- the subsequent metabolic processes
- the metabolism and elimination rate of the insulin molecule

The shift between changes in the blood insulin concentration and changes in the glucose is dependent upon the time that is required to complete the cascade of steps making up the cellular mediation of the insulin action. Once insulin has been absorbed into the bloodstream, this time is more or less constant. Furthermore, this cascade explains why insulin action can still be detected after the blood insulin levels have re-

turned to basal values. It also explains why identical blood insulin levels (as they can be established by means of an i.v. insulin infusion) can induce widely different metabolic effects, depending on the insulin sensitivity of the given subject. Not only can this sensitivity vary considerably between subjects, also the within-subject variations—induced, for example, by severe exercise—are pronounced.

PREVIOUS APPROACHES TO DETERMINE THE PROPERTIES OF INSULIN PREPARATIONS

The quantitative measurement of the pharmacokinetic and pharmacodynamic properties of insulin preparations has been performed by so-called "indirect" and "direct" methods.[10] Direct methods are considered those measuring the appearance of insulin in the blood or the insulin action. The indirect method, on the other hand, measures the signals of substances coupled to insulin.

Indirect method

With the indirect method, the pharmacokinetic properties of an insulin preparation are studied as follows: After test subjects receive s.c. injections of labeled insulin (^{125}I isotope, gamma emitter), the attenuation of the gamma radiation over the skin area above the insulin depot is measured using an external scintillation counter.[11] It is assumed that if the radiation activity measured above this skin area declines, this reduction is due to the absorption of labeled insulin. The result of the first measurement after insulin injection is defined as 100%, and the relative decline of radioactivity is plotted as the percentage of the arbitrary baseline value in a semilogarithmic representation over time. The reduction of radioactivity is considered to be a measure for the absorption rate of the injected insulin into the bloodstream.[12] Many studies of the pharmacokinetic properties of insulin preparations were and still are performed using this indirect method, since it can be carried out rather quickly and easily and it does not require the taking of blood samples or other invasive measurements.

There are two major areas of concern with this methodology: biologic and physical mechanics. From the biological perspective the absorption rate can only be inferred from the rate of decay of radioactivity of the injected insulin when the following three conditions are met[11]:

- The labeled insulin has the same absorption kinetics as the native hormone.
- The insulin molecule has to be absorbed unchanged, *i.e.*, intact, into the bloodstream.
- The measured radioactivity is proportional to the amount of non-absorbed insulin.

It is still under dispute whether the first condition is fulfilled, despite the development of monoiodinized insulin preparations, which are now also available for NPH insulins.[13] Experiments have shown that the second condition is not met, namely, a significant portion of the s.c. injected insulin is degraded and locally inactivated by proteolytic enzymes.[11,14,15] In addition to the degradation of insulin, the s.c. injected insulin is distributed irregularly in the s.c. fatty tissue. This irregular distribution is neither symmetric nor concentric and is influenced by the structure of the s.c. fatty tissue. Under these conditions, the radioactivity registered externally is unlikely to really be proportional to the total activity of the ^{125}I-labeled insulin below the injection site (third condition).

With respect to the physical/mechanical issues, the complex influences of measurement geometry and of the constancy of the scintillation counter ("quenching") have an unforeseeable impact on the results of the measurement. Depending on the counting characteristics of the gamma counter, the radioactivity of those insulin molecules that are closer to the surface is detected with a higher counter efficiency than that of the molecules that have penetrated into deeper tissue layers. The total efficiency of a scintillation counter can be defined as the ratio of the counting rate and the rate of decay. If and how many of the radioactive decays are recorded depends on a series of factors. The influence of the counter efficiency on the result of the measurement is one of these factors, *i.e.*, the ratio of the energy emitted as light and the energy released as radiation, is so great that an

interobservation variability occurs that has to be corrected for by obtaining standard counts prior to each measurement.

Thus the biologic and physical factors of the local degradation of insulin, the importance of the measurement geometry, and the efficiency of the scintillation counter, as well as the irregular distribution of the hormone administered by s.c. injection, make the results obtained with this indirect method clearly doubtful.

As an example, with the indirect method lower absorption rates (= longer half-lives) of the administered insulin preparations were measured than with other methods. A half-life of approximately 170 min following injection into the abdominal skin was reported for radiolabeled short-acting insulin.[11,16,17] This half-life of nearly 3 h is considerably different from the measured plasma insulin profiles and the metabolic activities of short-acting insulin preparations where maximal plasma insulin levels were observed after 90–120 min after s.c. injection, with a dramatic reduction of the blood glucose concentration by 30–60 min. During this time, however, up to 80% of the insulin injected is reported to be unabsorbed and present in the tissue by the indirect measurement method. The apparent contradiction between the slow disappearance of radioactive insulin from the injection site and the more rapid occurrence of peak plasma insulin concentrations may be explained by two factors. First, insulin absorbed into the blood is eliminated quickly with the maximum absorption rate of insulin (in percentage per hour of the injected dose) determining the peak plasma insulin concentrations. These concentrations are reached earlier than the measured half-life of the s.c. injected labeled insulin.[18] Second, the degradation of insulin molecules by macrophages/proteolytic enzymes in the s.c. depot leaving labeled insulin fragments contributes to the differences between the two methods of pharmacokinetic measuring, especially in the case of long-acting insulin preparations.

Metabolic activity is not the only outcome that calls the indirect method into question. The absorption rates determined by the indirect method exhibited a linear course for up to 5 h. The linearity of absorption is in contradiction with the exponential course of the absorption rates determined with ^{131}I measured in the serum and with the absorption kinetics calculated for s.c. injected substances.[19] This mathematically determined absorption rate could be confirmed in its monoexponential course in animal experiments using insulin labeled with ^{3}H, with the local insulin degradation in the tissue having been taken into account.[14]

Since the indirect method does not satisfactorily meet the premises required to evaluate the absorption kinetics of s.c. injected insulin, the indirect method is not suited for the quantitative investigation of the pharmacokinetic properties of insulin.

Direct methods

The direct methods allow the measurement of either the pharmacokinetic properties of an insulin preparation alone or the pharmacokinetic and pharmacodynamic properties simultaneously. The advantage of the direct methods, compared with the indirect method, is that the measurements are limited to only the insulin that has been absorbed into the blood or to the current metabolic activity of the insulin, respectively. Unfortunately, different methods and variations of these methods have been used in the various pharmacokinetic and pharmacodynamic studies of insulin preparations, which impede comparisons between the published results.[20] The information given for an identical insulin preparation can vary considerably; for instance, reports of the time to peak action of s.c. injected short-acting insulin vary between 0.75 and 4 h. Various efforts are presently being undertaken to come to standardized directives for the implementation of pharmacodynamic investigations of insulin preparations (see below).

One of the direct methods consists in measuring the plasma or serum insulin concentrations occurring after insulin injection (while the blood glucose concentrations decline) and in determining the pharmacokinetic properties from these measurements. The insulin concentrations reflect the bioavailability of each insulin preparation. The bioavailability is not only determined by the insulin absorption, but by the distribution and degradation of insulin

in the s.c tissue as well as the distribution and metabolism of insulin in the blood.

In subjects without metabolic disorders, serum C-peptide concentrations are measured in parallel with insulin concentration measurements to determine the proportion of circulating insulin due to endogenous insulin secretion versus exogenous insulin administration. A decline in the serum C-peptide concentrations after s.c. insulin injections demonstrates suppression of the endogenous insulin secretion.

In order to measure the pharmacokinetic parameters, the insulin concentrations are determined by means of assays utilizing antibodies that are able to recognize specific amino acid sequences (epitopes) of the insulin molecules. By labeling the antibodies with radioactive isotopes or with enzymes, it is possible to develop measuring systems that enable the quantitative determination of insulin in small amounts of plasma or serum with a high specificity. Such radioimmunoassays and enzyme-linked immunosorbent assays are commercially available. The majority of the antibodies used in the current radioimmunoassays recognize certain epitopes of the insulin antigen specifically, but they do not recognize whether the connecting peptide is still linked to one of the two chains of the insulin molecule (in the case of intermediate products of the conversion from proinsulin to insulin) or to both chains (in the case of proinsulin). Since these epitopes are identical for endogenous insulin and for exogenously administered human insulin, it is not possible to differentiate between these two insulins. The antiserum used for these determinations (a mixture of polyclonal antibodies) also detects proinsulin or intermediate products circulating in the blood, in addition to insulin; thus the term cross-reactivity is applied to the binding. The extent of this overestimation depends on the concentration of the insulin precursors and on the extent of cross-reactivity.

For the measurements of circulating insulin analogue concentrations frequently the same radioimmunoassay as for preparations formulated with human insulin had been used. Because of the changes in the amino acid sequence, the antibodies may bind less avidly to these insulin analogue molecules, depending on the epitope that the specific antibody recognizes. When measuring insulin analogues, these analogues must be used as standards for the construction of a standard curve, in order to account—at least to some extent—for the possible modification of the binding. A potential additional problem with regard to insulin analogues exists in the fact that differences in the affinity to the insulin receptor compared with human insulin and differences in the rate of metabolism may restrict the comparability of the measured results with those obtained with human insulin. These difficulties have to be considered in comparative statistical analyses of the results. The behavior of the rapid-acting insulin analogues insulin lispro and insulin aspart in terms of receptor binding and metabolism corresponds largely to that of human insulin.

Another variation of the "direct" method is to take blood samples at regular intervals that are not only used to measure insulin concentrations, but are also used to measure the blood glucose concentrations. The glucose values will show the pharmacodynamic effects of the insulin preparations under study. The following parameters serve to describe the effects: the time until the earliest measurable blood glucose-lowering effect ("time of onset of action"), the time until the maximum decline of the blood glucose concentration ("time to peak"), the blood glucose concentration at that time ("peak"), and the time until blood glucose concentration returns to baseline ("time of action"). However, the decline of blood glucose levels occurring with this experimental design can lead to hypoglycemic levels (<50–60 mg/dL), resulting in the stimulation of the release of insulin-lowering hormones, a counterregulatory mechanism of the body to increase the blood glucose concentration. Because of this mechanism, the repeated endogenous increase of blood glucose leads to an underestimation of the investigated insulin preparations' duration of action (see Fig. 2). Despite this difficulty, which aggravates the interpretation of the obtained pharmacodynamic data, all short- and long-acting insulin preparations had, because of their rather easy experimental practicability, been investigated by this method.[21]

To prevent this counterregulatory reaction induced by the insulin-induced hypoglycemia,

in the "Gerritzen's test" the subjects take in carbohydrates at regular intervals (biscuits or mashed potatoes; 10 g of carbohydrates/h).[22] This measure prevents hypoglycemia, but may stimulate endogenous insulin production, an effect that might invalidate the interpretation of the insulin concentrations measured in these experiments. This test is limited to the investigation of pharmacokinetic properties of insulin preparations only, since the blood glucose concentration varies continuously.

THE EUGLYCEMIC GLUCOSE CLAMP TECHNIQUE

During a glucose clamp the infusion rate of an i.v. glucose infusion (GIR) is varied according to regular measurements of the blood glucose concentrations in order to keep the blood glucose level constant. Therefore, the amount of glucose infused reflects the blood glucose-lowering effect of the applied insulin (Fig. 1). This experimental approach allows quantifica-

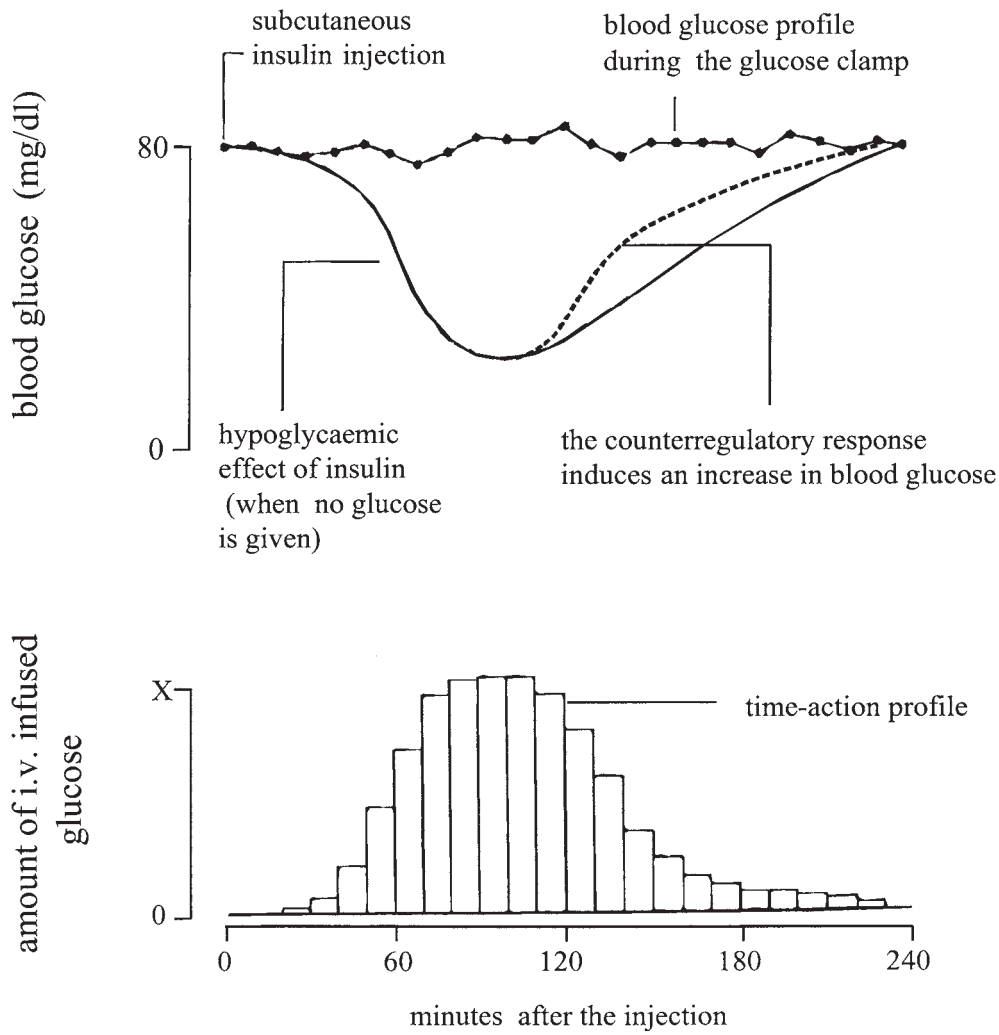

FIG. 1. Principle of the euglycemic glucose clamp technique.[23] After s.c. insulin injection, the blood glucose concentration decreases. After some time, the metabolic activity of the administered insulin decreases again, and the blood glucose level rises again. When the blood glucose level falls below the threshold at which a hormone secretion causing the blood glucose level to rise again is elicited (counterregulation), the blood glucose level increases more rapidly than one would expect from the action profile of the insulin preparation (dashed curve). When a decrease of the blood glucose level is prevented by an i.v. glucose infusion, *i.e.,* the blood glucose level is kept almost constant at baseline or a given target level (solid line with dots), the amount of glucose infused reflects the metabolic activity of the injected insulin at a given point of time, and the plotting of the glucose requirement over time reflects the time–action profile of the insulin preparation.

tion of the pharmacodynamic properties of the applied insulin.

In euglycemic glucose clamp experiments the GIR is calculated and varied to keep the blood glucose concentration constant at euglycemic values (normally 5 mg/dL below the subject's fasting glucose or about 80–100 mg/dL). The recorded GIR then constitutes a measure of the net effect of the insulin, *i.e.,* it describes the resulting sum of the decrease in hepatic glucose production and the increase in glucose utilization. By maintaining the blood glucose concentration constant a counterregulatory reaction is prevented.

Depending on the pharmacokinetic properties of the insulin preparation, the glucose requirement changes with a variable delay and to a variable extent after insulin administration. The GIR thus constitutes a quantitative parameter reflecting the metabolic activity of the investigated insulin preparations over time. The representation of the measured GIRs over time describes the time–action profiles of the insulin preparations. The insulin concentrations are determined from additional blood samples obtained during the experiments, and the pharmacokinetic properties of the administered insulin (s.c. or i.v. injected or oral/pulmonary insulin preparations) can be derived from the shapes of the insulin concentration–time curves.

This direct method currently represents the most suitable method to investigate both the pharmacokinetic and the pharmacodynamic properties of insulin preparations.

PROPOSAL FOR A STANDARDIZED GLUCOSE CLAMP TECHNIQUE

The use of a standardized glucose clamp technique for studies to estimate the pharmacokinetic and pharmacodynamic properties of insulin preparations or insulin administration techniques should lead to a better comparability of the results of such studies than has been the case in the past. The summary measures obtained by means of a uniform methodology would further have the advantage to help insulin-producing companies to make use of a comparable presentation form in order to describe their insulins. This would then permit physicians and patients with diabetes mellitus to compare the properties of different insulin preparations, which is especially important with the new insulin analogs. The following procedure is proposed for standardized glucose clamp experiments:

Study design

All studies—as far as possible—should use a double-blind design. The double-blind design is required in order to exclude an arbitrary or unconscious influencing—by whom- or whatever—of the study results. In order to facilitate the evaluation and also the comparison of the results obtained with new insulin preparations/administration forms, in all studies a study arm should be included in which preparations/administration forms are used that have already been studied.

Performing glucose clamp experiments

Glucose clamp experiments can be performed manually or automatically using a Biostator (Fig. 2). When using the manual glucose clamp technique the glucose concentration is determined from blood samples taken at regular intervals, and the GIR is varied according to the measurement results. This technique provides results similar to the automated method, as long as there are no rapid changes in the glucose requirement. If such rapid changes occur, they may not be recognized in time, depending on the length of intervals between the blood glucose measurements during a manual glucose clamp experiment.[25] The sampling frequency required to adequately recognize and describe the signal has to be based on the most rapid changes of the signal, *i.e.,* the velocity of changes in the glucose requirement. For this reason and because of the low ability of the examiner to influence the study results, comparative studies of insulin preparations should be performed using the automated glucose clamp technique.

An additional problem with the manual clamp technique is the higher blood loss when the glucose measurements are performed with standard laboratory methods. This method has also a considerably increased demand for man-

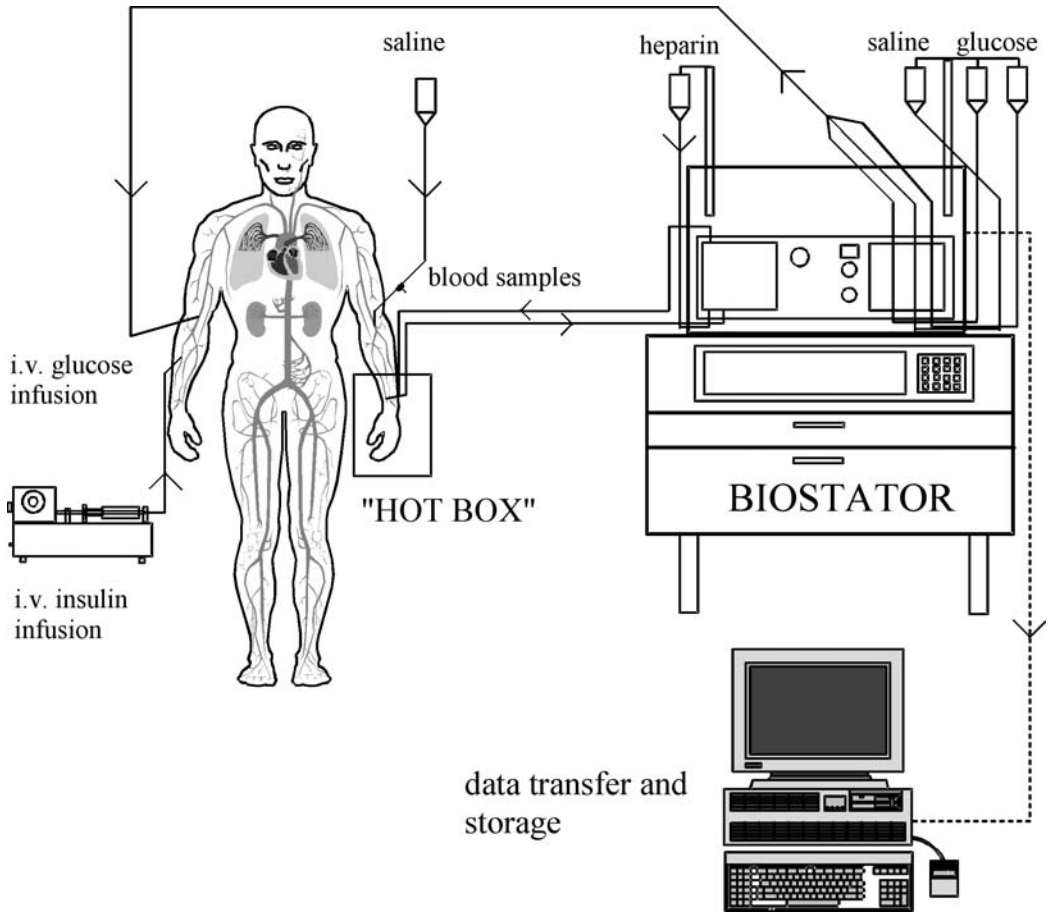

FIG. 2. Euglycemic glucose clamp technique using a Biostator.[24] The subject's hand is in a box with an air temperature of 55°C. Blood supplemented with heparin is continuously pumped from the hand vein to the Biostator. The i.v. glucose infusion given into the forearm of the contralateral arm is continuously adjusted to the current blood glucose concentration. Saline solution is continuously infused with a low flow rate in order to keep the venous access patent. A constant low i.v. insulin infusion is administered into the vein of the same arm to establish comparable basal insulin concentrations and to (partly) suppress the endogenous insulin production. Blood samples for blood glucose determinations and subsequent hormone measurements are taken from a cubital vein of the left arm at regular intervals. The results printed by the Biostator are, at the same time, stored in a computer for a later data analysis.

power, should greater numbers of these experiments be performed over longer periods of time. The manual performance requires a lot of experience with these experiments, namely, with regulating the GIR appropriately to keep the blood glucose closely at the target value. The manual performance, in principle, introduces the risk of a bias in the study if it is not performed blinded.

With a Biostator the blood glucose concentration is continuously measured, and it calculates the infusion rate of an i.v. glucose infusion by means of a direct negative feedback algorithm, which is based on the deviations of the measured results from a given target value. By this the blood glucose concentration is constantly kept as close as possible to the target value. For the calculation the glucose clamp algorithm (Mode 9), which is implemented in the Biostator, can be used. This algorithm takes both the currently measured blood glucose concentration and the extent of variations in the blood glucose concentration during the preceding minutes into account. The major disadvantages of the Biostator are its age and limited availability of machines. Furthermore, it ist difficult to maintain the system, the special tubings, etc., it requires are expensive and not easy to get, and it takes a lot of experience to run the Biostator. Up-to-date successors are cur-

rently under development in Europe and are available in Japan.

Performing glucose clamp experiments with the Biostator

For the continuous blood glucose measurement with the Biostator an i.v. needle is inserted retrogradely into a larger vein of the left hand of a test person/subject, and a double-lumen catheter is introduced into the needle, in order to enable a continuous withdrawal of blood. One of the two tubes of the double-lumen catheter is used to pump a heparin-saline solution (50 IU of heparin/mL of saline) into the i.v. needle. The other tube is used to continuously drain the same amount of venous blood, which is mixed with the heparin solution.

An arterialization of the blood withdrawn via the double-lumen catheter is achieved by leaving the subject's left hand in a so-called "hot-box" during the entire duration of the experiment. A thermostat switch ensures an air temperature of 55°C in this box by activating and inactivating a heating system. The warming of the hand causes the arteriovenous anastomoses to open, with the consequence that part of the arterial blood flows directly into the venous system. The arterial blood glucose concentrations mirror the processes in the central compartment, and, thereby, reflect the degree of glucose utilization of all tissues or the glucose supply by the hepatic glucose production or by glucose absorption in the splanchnic region without being influenced by local conditions. The blood glucose concentrations in the sampled venous blood are comparable with those in the arterial blood. The described efforts in taking blood samples for the continuous blood glucose measuring are undertaken because—depending on the prevailing insulin concentrations and the degree of activity of the musculature—the glucose concentration in the venous blood is lower than in the arterial blood due to the glucose uptake of the musculature.

Subjects

We propose to examine healthy subjects of normal weight in the range of 18–45 years with a body mass index of <27 kg/m^2. Overweight subjects should not be examined, since they tend to be insulin resistant. The subjects must be free from acute or chronic diseases, and none of them should be receiving any pharmacotherapy (with the exception of chronic medications that are known not to influence insulin sensitivity). All subjects first must undergo a thorough medical examination, including an electrocardiogram. This is not only to ensure the subject's safety in view of the stress related with the investigations, but also to exclude the existence of other diseases that influence insulin sensitivity (such as thyroid disease or infection) as far as possible. Those clinical chemistry parameters that determine liver and kidney function especially should be within the reference ranges, since physiological function of these organs is crucial for the metabolism of insulin. The aim of these inclusion and exclusion criteria is to obtain a largely comparable subject population for the studies.

Studies can also be performed with patients with diabetes. If patients with Type 1 are studied it is mandatory to establish comparable baseline blood glucose and insulin levels for some time prior to the intervention (= insulin application) in order to achieve comparable baseline conditions on all study days. As the results of such experiments not only the glucose consumption over time is presented, but also changes in blood glucose are given. When the metabolic activity of the applied insulin is vanishing, blood glucose starts to increase. The rapidity of this increase in blood glucose and the time until certain limits were reached are also a description of the properties of the applied insulin preparation (see below). It is also possible to calculate a composite curve taking both the glucose consumption and the blood glucose values into consideration at the same time (= activity profiles).

Study days

Prior to each study day the subjects should follow certain rules in order to achieve at the various study days an intra- and even interindividually comparable insulin sensitivity. The subjects are, *e.g.*, told to refrain from unusual physical exercise and excessive alcohol consumption the day before the experiments.

The subjects are instructed on the morning of the study day, to avoid exercise (such as the use of a bicycle to come to the clinic), that they should not drink coffee, tea, or other caffeinated beverages, and that they are not allowed to smoke. Ideally the subjects should be admitted to the research center the evening before the study.

The subjects should be studied in the fasting state; they should have taken their last meal >12 h ago. The study days should always start at the same time, *e.g.,* at 8 a.m. The subjects should stay in bed throughout the experiment and receive only mineral water *ad libitum* but otherwise remain fasting. The total blood loss by blood withdrawal should not exceed 500 mL during the studies. Because of the blood loss, all tests performed in the same subject should be separated by at least 3 days.

Gender of the subjects

So far, women usually have not participated regularly in glucose clamp studies as subjects, although this would have been reasonable in order to evaluate whether the substances under study act in a comparable manner in both sexes. There are several reasons why this has not been done in the past. When novel drugs, *e.g.,* insulin analogues, are studied, there are usually no data available about their possible harmful effects on the unborn at the time of the implementation of these early studies (phase I studies). If, nevertheless, women participate in such studies, non-pregnancy and adequate contraception have to be ensured.

It is uncertain whether a woman's insulin sensitivity varies, depending on the point of time in her menstrual cycle, to such an extent that relevant changes in the results of measurements could occur. The results of the studies thath had been performed to elucidate this question are contradictory.[26–33] If the menstrual cycle has a relevant influence on insulin sensitivity, the investigations ideally should always be performed at the same time of the cycle. Since there must always be at least 3 days between the experiments, the most unfavorable consequence would be that only one experiment per cycle could be performed. If several experiments are to be performed, this would mean a long study duration. During this time other effects with influence on insulin sensitivity may occur, such as differences in physical activity, depending on the season. Despite these problems, women should participate as subjects in future studies, in order to obtain relevant information about the properties of the substances under investigation early on.

Insulin dose

Before insulin is applied, a basal phase of 2 h should be included in order to allow the subjects to adjust to the experimental situation and to establish a comparable metabolic situation in terms of insulin, catecholamine, and other hormone concentrations. During this phase the basal glucose requirement can be determined.

Normally, the insulin doses applied should not grossly exceed the therapeutically reasonable range that is commonly used in clinical routine, *i.e.,* >0.2 U/kg of body weight for short-acting insulin or >0.3 U/kg for long-acting insulin. To determine an equipotent dose for insulin analogues or other routes of insulin administration is, however, difficult, and a value/dose can only be approximated, since the preliminary data from animal experiments or small pilot studies provide only limited information for humans. Depending on the metabolic effect of each insulin preparation, equimolar dosages may not necessarily yield comparable effects. When extremely high insulin doses are applied, high amounts of glucose (= amounts of liquid) have to be infused in order to keep the blood glucose concentration constant, so that the technical feasibility of the studies will be aggravated by frequent interruptions in order to go to the lavatory, worsening at the same time the quality of the glucose clamp experiments (= of the study results).

Site and mode of injection

In the case of abdominal s.c. injection this should be given into a lifted fold of skin 5–10 cm to the left or right of the umbilicus in the direction of the iliac crest. All injections should preferably be performed by the same experienced investigator in order to ensure a reproducible s.c. injection. Care should be taken to

keep the modalities of the injection technique constant (injection angle, depth of injection, and velocity of injection).

For s.c. injections insulin syringes with a small dead volume should be used. To delay insulin absorption, long-acting insulin preparations are frequently injected into the thigh. Experimental results obtained with short-acting insulin and long-acting insulin can only be compared if the same injection site is used, as otherwise the lag effect associated with the injection site, which is difficult to estimate, would have to be taken into account. In long-acting insulin preparations, which are suspensions, a good and comparable homogenization has to be achieved by thorough shaking before the required dose can be drawn up.

Endogenous insulin production

During the experiments the endogenous insulin production must be suppressed. Otherwise, the observed glucose requirements cannot be attributed solely to the trial insulin injection, and could, in part, be due to the effect of endogenously secreted insulin. Similarly, the pharmacokinetic insulin measurements could be misleading because of the combination of exogenous and endogenous insulins being measured by the assay. The extent of the endogenous insulin production can be estimated by measuring the serum C-peptide concentration, but this is not a good substitute for suppressing endogenous secretion.

In order to allow a reproducible quantitative measurement of the pharmacodynamic properties of s.c. injected insulin preparations, a comparable baseline insulinemia has to be established. The serum insulin concentrations are raised to comparable values by a constant low-dose i.v. insulin infusion (*e.g.*, 0.15 mU/min/kg of body weight) in the different subjects and on the different study days. Thus, a comparable suppression of the endogenous insulin production is achieved without provoking a peripheral hyperinsulinemia.

In connection with the baseline i.v. insulin infusion a suppression of the endogenous insulin secretion takes place. Serum C-peptide concentrations should be estimated in order to make sure that this suppression remains constant during the experiments. Because of the longer half-live of C-peptide, the measurement intervals can be longer than with serum insulin. The serum C-peptide concentrations fall by 20–30% prior to the s.c. insulin injection. After s.c. insulin administration, the values fall by an additional 10–20% and remain at these low levels during the experiments. With declining blood insulin levels towards the end of the experiments, the serum C-peptide concentrations may return (= increase) to the preinjection values. With this procedure in place, it can be ensured that the observed changes in serum insulin concentrations and the GIRs measured after insulin application are predominately due to the absorption of the exogenous insulin. Setting the target glucose level below the patient's fasting glucose also helps suppress endogenous insulin production.

Blood glucose target value

The target value of blood glucose should be identical in all experiments, since the glucose requirement is influenced by that target value. Therefore, the individual basal blood glucose should not be used as the target value. In order to get a uniform (and thereby comparable) blood glucose level, the suggestion is to keep it constant at 90 mg/dL during the experiments.

CRITICAL DISCUSSION OF THE GLUCOSE CLAMP TECHNIQUE

This technique had been criticized with the argument that the endogenous glucose production is only partly suppressed and that by this, depending on the current insulin concentrations, the extent of suppression is changed.[34,35] Therefore, following this argument, the recorded GIRs that are required to maintain euglycemia could only allow a gross evaluation of the glucose metabolism but no quantitative determination.

This criticism is based on the fact that the hepatic glucose production is suppressed to a variable extent depending on the established blood insulin level.[36] The basal hepatic glucose production amounts to 2.0–2.5 mg/min/kg in

fasting adults.[36,37] From the experience in our own (L.H.) experiments it can be said that a GIR of 1.0–2.5 mg/min/kg is required during the basal phase (with serum insulin concentrations of 10–15 μU/mL) in order to keep the blood glucose concentration constant. Assuming that the exogenous glucose infusion replaces the endogenous glucose production, it can be concluded that the hepatic glucose production is almost completely suppressed during this phase of the experiment. This corresponds to reports in the literature that insulin concentrations as low as 10–15 μU/mL can be expected to cause a suppression of the hepatic glucose production by more than 30% (50% suppression at approximately 30 μU/mL).[36–38] It is well known that after s.c. injections of, for example, short-acting insulin, the insulin concentrations increase to maximal values of 40–60 μU/mL. These concentrations lead to an accordingly stronger, almost complete suppression of the hepatic glucose production. This suppression will decrease during the course of the experiment as blood insulin levels return toward baseline values. The variable suppression of the hepatic glucose production during the experiments is part of the processes that occur in patients with diabetes mellitus as a physiological response to the exogenous insulin administration. The GIR compensates for the difference of the peripheral glucose utilization rate and the hepatic glucose production, resulting from the prevailing blood insulin level at the specific times after s.c. insulin injection. This varying glucose requirement of the body is determined by the measurement of the glucose flux by the glucose clamp technique. The objective of this methodological approach is to determine the physiologic action profile of s.c. injected insulin preparations. A complete suppression of the hepatic glucose production, *e.g.,* by a higher basal blood insulin level, is neither necessary nor desirable, as the aim of the described setting is to create a situation that is as physiological as possible. If required, information about the exact proportion of the hepatic/kidney glucose production and of the peripheral glucose metabolism can be obtained by glucose turnover measurements using glucose molecules marked with radioactive isotopes or stable isotopes.

In subjects without metabolic disorders the described experimental method, by means of the infused glucose, prevents stimulation of endogenous insulin secretion and suppresses hepatic glucose production. Methodological alternatives to this experimental methodology are:

- injection of high insulin doses of up to 0.5 U of insulin/kg of body weight[39–42]
- infusion using high i.v. insulin rates in order to establish a high basal blood insulin level
- constant maintenance of the blood glucose concentration at values of 65 mg/dL[43]

For the first two methodological alternatives it is true that the endogenous insulin production and the hepatic glucose production are further suppressed. However, due to the resulting hyperinsulinemia, there may be stimulation of peripheral glucose utilization. The third methodological alternative may produce a secretion of counterregulatory hormones when blood glucose falls below a certain, interindividually varying threshold, even with blood glucose concentrations maintained around 65 mg/dL. The secretion of, *e.g.,* cortisol and adrenaline leads to a rapid and dramatic worsening of insulin sensitivity. Therefore, these methodological alternatives cannot be used for pharmacological investigations of insulin preparations under conditions reflecting normal physiology as far as possible.

In the case of longer-lasting glucose clamp experiments (>5 h) with constantly increased blood insulin levels, the peripheral glucose utilization had been reported to increase because of an alteration of the insulin sensitivity during the experiments.[44] During these experiments, nonphysiologic, constant plasma insulin concentrations of 100 μU/mL had been established, which were twice to 10 times as high as those achieved temporarily in investigations designed to study the pharmacodynamic properties of insulin preparations or different insulin administration techniques.

Complete suppression of endogenous insulin production can also be achieved by continuous infusion of somatostatin. This method results not only in a total suppression of beta-cell activity, but also in a simultaneous sup-

pression of alpha-cell activity. Consequently, to restore a normal hormone situation, glucagon—and other hormones—must be replaced. Moreover, somatostatin provokes a suppression of the perfusion of the splanchnic nerve area (leading to a slowed absorption from the intestine) and a slowing down of stomach emptying.[45] For pharmacological investigations of insulin, it is important to note that somatostatin reduces the insulin clearance by 20%, with the duration of insulin action thus being prolonged artificially.[46]

QUALITY OF THE PERFORMANCE OF THE GLUCOSE CLAMPS

Until now, there were no definite criteria that, in the manner of a "quality control," would allow one to decide whether a glucose clamp was successful or not. Should prolonged technical problems occur during the conduct of an experiment (loss of results of measurements >10% of the duration of the experiment) that might possibly be associated with a major divergence of the current blood glucose level from the target value, the experiment should be stopped. It should also be stopped and repeated if the blood glucose should fall to hypoglycemic values (<60 mg/dL), since in that case the insulin sensitivity cannot be expected to be comparable at different study days.

One possible measure to describe the quality of glucose clamp experiments is to state the coefficient of variation [CV; defined as (standard deviation/$\bar{\chi}$) × 100] of the recorded blood glucose concentrations. As a relative, dimensionless measure of dispersion, the CV provides information about the variability of a measurement, irrespective of the order of magnitude of the figures. Usually, a CV of the blood glucose concentration of <5% is considered to be a criterion for a successful glucose clamp of sufficient quality. However, this interpretation ignores the fact that the time–action profile of the insulin preparation under study determines the degree to which the glucose concentration can be kept constant. When dramatic changes in the glucose requirement occur rapidly, it is more difficult to keep the blood glucose concentration constant than during relatively stable glucose requirements. Another possibility to describe the uniformity of the blood glucose level over time (this dimension is missing in the CV and in frequency diagrams) is the so-called CUSUM (= cumulative sums) representation.[25]

CHARACTERIZATION OF TIME–ACTION PROFILES: VARIATIONS OF THE RECORDED GIR VALUES

The adaptation of the GIR to the current glucose requirement is made on the basis of the blood glucose concentration measured either every minute (Biostator) or at more or less long intervals (manual clamp). Because of the regulation times in the closed loop, a change of the GIR does not immediately induce a change in the blood glucose concentration. Therefore, the blood glucose concentrations usually do not correspond to the exact target value but vary around it. In response to that, variations of the GIR occur. These variations (= noise) aggravate the analysis of the GIR profiles (= signal). Depending on the amount of noise, a determination of summary measures (see below) from the original data may be difficult. For example, a single massive variation in the GIR profile might be interpreted as peak metabolic activity, although this does not correspond to the global shape of the curve. The reasons for such variations in the GIRs, which may be randomly distributed over the duration of the experiment, can be technical problems such as malfunctions in the continuous blood glucose measurement in the case of the Biostator.

A rapid adaptation of the GIR to a changing glucose requirement (with a threatening decrease of blood glucose) presupposes that the regulation algorithm responds even to a rather small decrease of the blood glucose level with an increase of the GIR. The extent of the delay occurring with the glucose clamp algorithm used by the Biostator is approximately 5–10 min, although the feedback algorithm takes the velocity of the change in the blood glucose concentration into account. The slowness of this control impedes a timely adaptation of the GIR in the case of rapid increases in the glucose requirement, leading to a short-term decline of the current glucose concentration below the target value. Thus, a few minutes after the in-

jection of, *e.g.*, rapid-acting insulin analogues, a mean deviation by <5 mg/dL from the target value of 90 mg/dL will/can occur over a period of about 10 min. Nevertheless, the manual glucose clamp technique, with its even greater intervals between blood glucose measurements, has at least similar difficulties in keeping blood glucose at the target level in such situations.

The noise of a recorded signal can be reduced by various measures. The available smoothing techniques may lead to a distortion of the current signal. The use of moving averages can introduce a shift of the time–action profile to the right on the time *x*-axis, since increasing GIR values provoke an increase of the mean value only after several arithmetic steps. By fitting a mathematical function to the original measured signal (see below), the noise in the GIR profiles can be suppressed if all available data are taken into account. The variations in the GIR profiles induced by artifacts are not interpreted as measurement signals when fitting a function to the total profile.

FITTING OF A FUNCTION TO THE TIME–ACTION PROFILES

The recorded GIRs describe the metabolic activity of the administered insulin preparation (= time–action profiles). To characterize unimodal time–action profiles, as they are typically obtained with insulin preparations, the following measures ("summary measures") appear to be the most relevant, since they characterize the time–action profiles that are most important for insulin therapy in clinical practice (Fig. 3). GIR_{max} represents the maximal GIR required to maintain the blood glucose con-

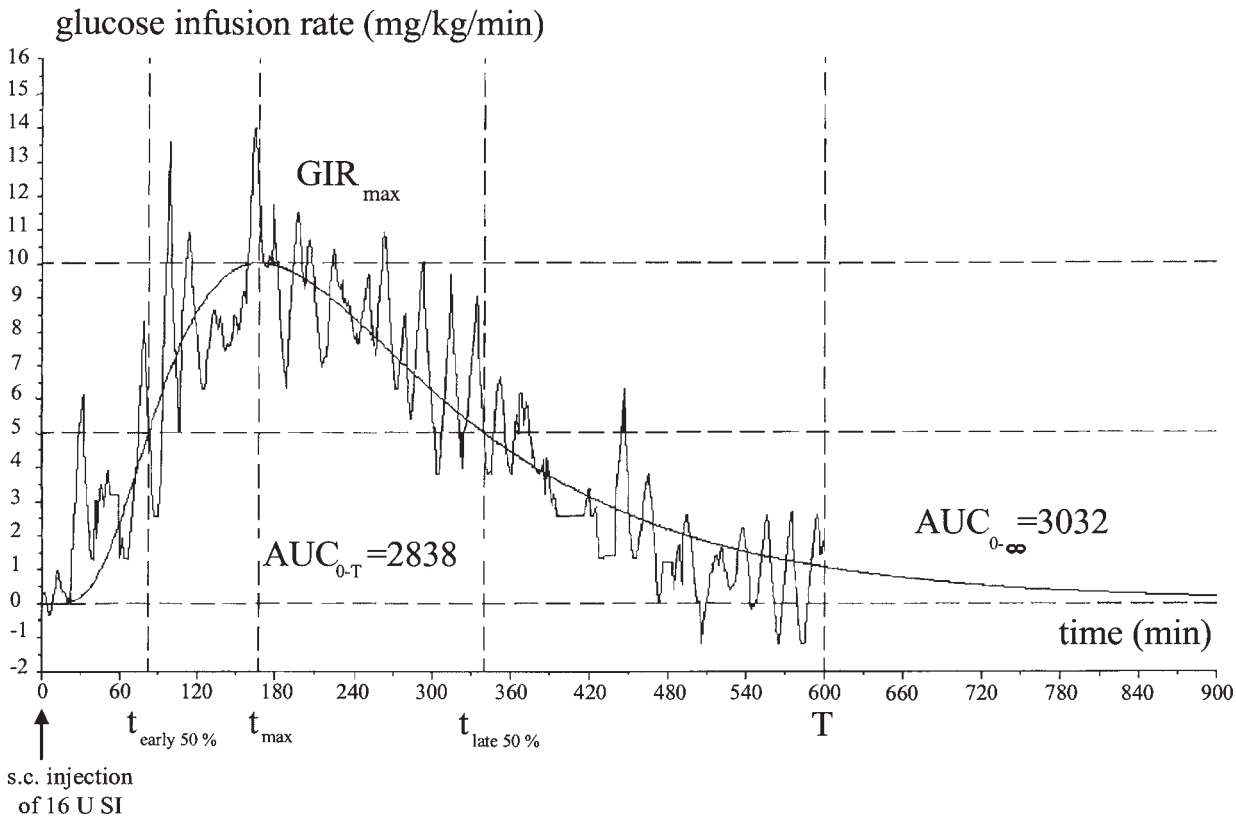

FIG. 3. Time–action profile with certain summary measures. GIR_{max} represents the maximal GIR required to maintain the blood glucose concentration at the desired target value, considering the variations of the original values during the experiments. The time point after s.c. injection at which GIR_{max} was achieved is described by t_{max}. The time points at which 50% of the GIR_{max} occur before (early $t_{50\%}$) and after (late $t_{50\%}$) the GIR_{max} determine the rate of increase/decrease of action. The area under the action profile (AUC) can be calculated either for the entire action profile, *i.e.*, until the latter returns to the zero line ($AUC_{0-\infty}$), or just for the period of the experiment or for shorter intervals (AUC_{0-T}). SI, short-acting insulin.

centration at the desired target value, considering the variations of the original values during the experiments. The time point after s.c. injection at which GIR_{max} is achieved is described by t_{max}. The time points at which 50% of GIR_{max} occur before (early $t_{50\%}$) and after (late $t_{50\%}$) the GIR_{max} determine the rate of increase/decrease of action. The area under the time–action profile [area under the curve (AUC)] can be calculated either for the entire action profile, *i.e.*, until it returns to the zero line ($AUC_{0-\infty}$), or only for the period of the experiment, or for shorter intervals (AUC_{0-T}). Depending on the shape of the measured profiles, various summary measures may be useful. Because of the relatively slow onset of action and offset of action it can be difficult to precisely define such time points. This also hampers the estimation of the duration of action.

The use of these summary measures has been recommended in the literature for the description of results from serial measurements in order to adequately describe the most important properties of such curves and in order to be able to apply adequate methods of statistical analysis (see below).[47] The purpose of the determination of summary measures is to describe comprehensible and interpretable characteristics of the time–action profiles by using as few figures as possible, with these figures having been determined for each individual subject or for each individual experiment. The raw data themselves (in this case the originally measured time–action profiles) do not undergo statistical analysis, but in this two-stage approach the summary measures are determined first and then in a second step analyzed by appropriate statistical procedures (see below).[47] The advantage of such summary measures is that the great number of measuring results is reduced considerably, without at the same time losing important information that had been collected in extensive studies.

The summary measures can be determined either directly from the measured time–action profiles or after the application of adequate smoothing methods to the profiles. Another possibility is to fit an appropriate function to the time–action profiles. The time–action profiles obtained can be adequately described by the fitting of simple polynomials or (more complexly) by the fitting of nonlinear regression models.[48]

The advantage of fitting a log-normal function is that it takes overlaid autocorrelated confounding factors into consideration.[49] The time–action profiles must have the following characteristics in order to allow a successful fitting of the proposed function: a relatively rapid increase, followed by a slower decrease after the single peak, both with many measurement points. Usually, the basal GIR is subtracted in order to fit the function. The decisive advantage of such a fitting of a function is that the entire relevant information on the time–action profile is summarized in three function parameters. Although this log-normal function has only three parameters, it can be adapted to a great number of unimodal curves of various shapes. The summary measures used to describe the time–action profiles can simply and also definitely be calculated by means of the three parameters of the regression function. However, if the profiles exhibit a very rapid onset of action (as it is often the case with inhalation of insulin) or a slow onset of action (as it is the case with long-acting insulin preparations) this function does not allow an adequate fitting.

STATISTICAL ANALYSIS OF TIME–ACTION PROFILES

A complex statistical approach to the analysis of time–action profiles, known as the analysis of variance (ANOVA) for repeated measurements, is quite frequently used when measurements of the same parameters are to be performed in the same subject over a certain period of time. However, this method cannot be reasonably used if:

- the number of measurement times is significantly large
- the number of subjects is comparably small
- several "within-subjects" factors are present

These conditions are almost always present in studies with multiple administrations of different insulin preparations or dosages on different study days and with long duration ex-

periments with many measurement values. In the ANOVA methods for repeated measurements (with ε adjustment of the degrees of freedom) the number of degrees of freedom is insufficient under the mentioned conditions, which means that no meaningful statistical analysis can be performed. Another inconvenience of this approach is that an interpretation of the results is difficult and that medical questions are not answered in a satisfactory manner. Originally, these methods had been developed for the analysis of short time series of measurements at only three to 20 different measurement times. Since the ANOVA approaches are not usable for the analysis of GIR profiles, which involve a greater number of measuring times (= long-time series), the summary measures (or the parameters of the fitted function) can be used for statistical analysis. The study hypothesis underlying the studies can then be analyzed using simpler methods of ANOVA (such as the two-factorial ANOVA for a randomized block design). The fitting of functions, with a subsequent determination of summary measures and statistical analysis, is only adequate if the functions describe the shape of the original curves so well that the summary measures can be determined with sufficient precision. Therefore, each individual GIR profile including the fitted function has to undergo a visual inspection to check the quality of the fitting.

GROUP AVERAGE AND TREATMENT AVERAGE

The results of a study can be expressed as a series of individual time–action profiles with fitted functions. Usually, the arithmetic mean of the time–action profiles is then calculated for one insulin preparation and together with information on the dispersion measure, which usually is the standard error, is plotted in the figures. Now, the question is how closely this can mean curve express the typical shape of the individual time–action profiles. The arithmetic mean is calculated from the GIR values recorded at a specific point of time after insulin injection. The mean values of the various time points, the so-called treatment mean values, are plotted against the time and describe the action profile of the insulin preparation under investigation. The resulting shape of the curve does not necessarily reflect the typical shape of the individual curves.[50] The mean value curve resulting from the calculation of the individual curves (reaching their peaks at different times) is usually flatter and broader than the individual curves.

If a function is fitted to the individual curves and then the summary measures of this function are averaged, the resulting values can describe the basic shape of the individual curves better than the arithmetic mean curve. This also explains why the arithmetic mean (a group mean) to describe the results obtained with each insulin preparation obtained in the individual experiments, is not identical with the treatment mean value, as read from the figures.

PROBLEMS WITH THE TIME–ACTION PROFILES OF LONG-ACTING INSULIN PREPARATIONS

When recording the time–action profile of a long-acting insulin preparation, it is difficult to assess, in the case of slowly declining insulin action, whether the glucose requirement existing at a specific point of time can still be attributed to the effect of the s.c. injected insulin or to the basal i.v. insulin infusion. Because of the variations of the GIR it is often not possible to fix a definite point of time for the end of the insulin action without fitting a function. Information about the duration of action of long-acting insulins is therefore always of poor precision. Nevertheless, the time at which the action falls to 50% or 25% of the peak action can be determined with a rather high precision.

The assumption that in glucose clamp studies the GIR returns to basal values (or zero) when the elevation of the blood insulin level following the administration of insulin returns to preinjection basal values is based on the hypothesis that the basal glucose requirement remains constant over a period of time with a given blood insulin level. It is known that, physiologically, fasting over a period of 36 h leads to an increase in insulin sensitivity. The identical basal blood insulin level, as a conse-

quence of the constant i.v. insulin infusion, may then lead to a higher metabolic activity. Therefore, it is difficult to decide whether the metabolic situation (= insulin sensitivity) in glucose clamp studies with a duration of 24 h allows the situation at the start of the experiment (after a 12-h fast) to be comparable with that at the end of the experiment (after 36 h without intake of solid food). During glucose clamp experiments with such duration, however, the subjects are not fasting in the original meaning of the word, because they receive i.v. infusions of up to several hundred grams of glucose per experiment, depending on the insulin preparation and the dose under investigation. With respect to carbohydrates the subjects are not in a state of fasting, but only with respect to proteins and fats that would otherwise be taken in with food.

The implementation of a control experiment, in which no insulin is injected s.c., but a basal i.v. insulin infusion is established, does not provoke a comparable metabolic situation to glucose clamp experiments, since under this condition much less glucose is infused. However, in the two studies in which such a control experiment had been included (one over 19 h, the other over 30 h) we have observed a constant GIR (= basal rate) over time.[51]

Since the glucose clamp experiments are conducted over a period of 24 h from one morning to the next morning, it is also necessary to take the influence of endogenous chronobiological cycles on the insulin sensitivity into account. Insulin sensitivity is known to be reduced in the early morning hours especially because of an increased secretion of growth hormone (dawn phenomenon). With a given blood insulin level, this might lead to a lower glucose consumption in the early morning hours. However, in most investigations with long-acting insulin preparations a return to basal GIRs was recorded. Thus, the insulin sensitivity does not seem to change regularly during the experiments. Other authors also report no increase in glucose consumption at the end of their experiments conducted over a period of 40 h.[42]

As mentioned before, because of the shape of their time–action profile it is difficult to indicate the time and the degree of the peak action of long-acting insulins and long-acting insulin analogues. Therefore, a characterization of their time–action profile is problematic. With long-acting insulin analogues, which exhibit an almost constant action over time (rectangular profile), information about the peak action makes little sense at all.

In the face of the problems described for the investigation of long-acting insulins, combined with the considerable variability of the insulin action of long-acting insulins, the detection of statistically significant differences in the duration of action or other summary measures between various long-acting insulins requires a large sample size of subjects.

AUC VALUES

The AUC of a concentration–time profile (pharmacokinetic measures) or time–action profile (pharmacodynamic measures) can be calculated for the entire area below the profile from 0 to infinity ($AUC_{0\text{-}\infty}$), or for a certain interval $[0,T]$, with T being any point of time, *e.g.,* the entire duration of the experiment or a specific period of time after the s.c. insulin injection. This partial $AUC_{0\text{-}T}$ can be calculated by integrating a fitted function or, for a recorded original profile, by using the trapezoidal rule. The calculation of an AUC over various periods of time after insulin application can be useful in order to describe the rapidity of changes in blood insulin levels and, thereby, in glucose requirements.

Since the determination of the time of onset of action defined as a certain increase in the glucose requirement or the determination of the duration of action of each insulin preparation is an arbitrary determination, the definition of thresholds may be useful. Waldhäusl[52] suggested *the onset of action* be defined as the point of time at which—after subtraction of the basal rate—5% of the total AUC has been reached and *the end of action* as the point of time at which 95% has been reached. Another way of analysis lies in the summation of the recorded GIRs over time. This method shows within which period of time after injection which amount of metabolic activity has been reached and when 100% of the activity has been

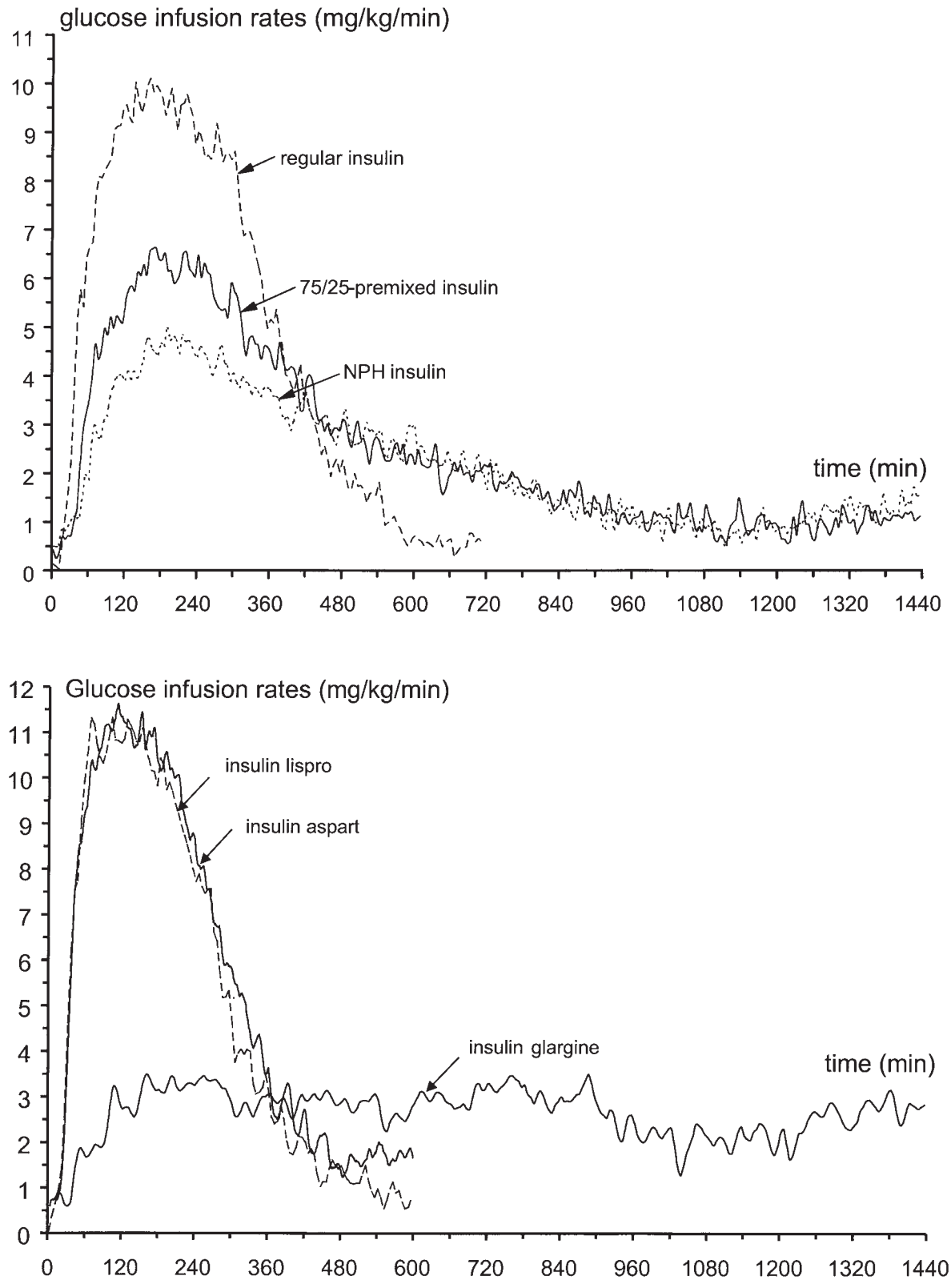

FIG. 4. Comparison of the time–action profiles of s.c. applied regular insulin and NPH insulin[4] (**a**) and of insulin lispro,[4] insulin aspart (unpublished data), and insulin glargine[4] (**b**). The data combined here in one figure originate from different studies; however, all were performed with the same glucose clamp technique in comparable groups of healthy subjects. The insulin dose applied was 0.3 U/kg of body weight, with the exception of 0.4 U/kg with insulin glargine.

achieved. If the suggested limits were used, this would also allow the estimation and comparison of the duration of action of different insulin formulations.

The calculation of the AUC is an attempt to obtain a summary measure enabling a simple comparison between various concentration–time curves or GIR curves. It has to be taken into account that AUCs of the same size may be obtained although the shapes of the curves can differ considerably. On the other hand, differences in the basal values may be of such an influence on the AUC that significant differences occur, although the shapes of the curves are identical. When the focus of an investigation is limited to the action of the applied insulin, the analysis can be restricted to the incremental AUCs, which would mean that the averaged basal rate has to be subtracted from all values recorded after insulin application. This procedure, in turn, may become problematic should the measured original values fall below the basal values, *i.e.*, negative areas occur.[50]

DETERMINATION OF PHARMACOKINETIC SUMMARY MEASURES

The following pharmacokinetic summary measures can be determined from the concentration–time profiles after subtraction of the basal rate, either directly or after fitting of a function: basal concentrations (C_{basal}), maximal concentration (C_{max}), time at which the maximal concentration is achieved (t_{max}), and time at which the early and the late half of the peak concentration are obtained (early $t_{50\%}$ and late $t_{50\%}$). Furthermore, the insulin concentration at the end of the experiment may be of interest (C_{end}). As mentioned above, the area under the profile (AUC) can also be calculated for various time intervals using the trapezoidal rule.

SUMMARY AND OUTLOOK

In summary, the proposed procedure for the performance of glucose clamp studies, which aim at evaluating the pharmacodynamic and pharmacokinetic properties of insulin preparations/insulin administration forms, allows the performance of such studies under standardized conditions. If this approach were generally used, a much better comparability of the results of such studies could be achieved. In the last 15 years we have performed a series of individual clinical studies investigating a wide variety of questions. As an example, the data shown in Figure 4a show the time–action profiles of the most widely used insulin preparations, and those in Figure 4b show those of the rapid-acting and long-acting insulin analogues available.

We do not believe that the proposed approach is ideal; however, with knowledge of the limitations and disadvantages, it appears to us to be the best methodology available until the development of reliable, implantable glucose sensors that will allow the study of the metabolic effect of insulin preparations under more clinically meaningful conditions.

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