Hormone and Metabolic Research

Volume 6/1974

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Georg Thieme
Verlag
Stuttgart

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Group Experiments on the Radioimmunological Insulin Determination*

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Summary

By means of two group experiments, performed in seven laboratories and using six different modifications in the insulin radioimmunoassay technique, it is shown that the use of standards diluted in human serum of low basal insulin concentration, instead of buffer, improves appreciably the interlaboratory precision. The causes lies perhaps in some serum factor, which could disturb some modifications of the radioimmunoassay more than others. There was no improvement of precision when the same insulin standard was used in all laboratories. Standard curves made up in different human fasting sera have a scatter only within the range of pipetting and counting error.

Key-Words: *Group Experiment – Insulin RIA – Data Processing – Quality Control – Insulin Recovery*

Introduction

Elimination of inter-laboratory differences of the results of clinical chemical analyses has recently drawn major attention. Quality control of routine methods largely is performed by means of participation in group experiments. The aim of this work was to design and to test a modell for collaborative, comparative studies on the radioimmunological insulin determination with the goal of improving the inter-laboratory precision.

Material and Methods

All experiments were performed on the same samples by seven individual groups of workers in different laboratories.

First group experiment (1972): For the first group experiment we dispatched the following two lots of serum samples to all seven participants.

Twentyfour samples from a serum pool, mixed from the serum of 5 patients, venipuncture one hour after breakfast.

Six serum samples with different concentrations of insulin, taken from one patient during an oral glucose tolerance test (OGTT). The venipunctures were performed at 0, 5, 10, 20, 30 and 60 minutes after the administration of 100 gm glucose. Each sample was assayed in triplicate. In addition, each box with the frozen samples contained a vial of 2 nU insulin standard preparation (kindly supplied by Fa. Buchler, Braunschweig – RCC, Amersham). With this insulin standard each participant prepared a standard curve in his own buffer system, and using his usual concentrations. The unknowns were then evaluated with the laboratory's own standard curve, as well as with the standard curve containing the common insulin standard.

Second group experiment (1973): The samples assayed in the second group experiment were as follows:

Nine samples from a new serum pool.

Triplicates of seven serum samples drawn from a further patient during an OGTT. Times of venipuncture: 0, 5, 10, 15, 20, 30 and 60 minutes after administration of glucose.

Triplicates of 8 samples to form the recovery curve. These were made in human serum with a low basal concentration of insulin. For this we used the serum of a fasting patient which contained approximately 6 μU insulin/ml. 200 μU insulin were then dissolved in each millilitre of an aliquot of this serum, so providing a stock serum standard of 206 μU insulin/ml. From this, further serial 1 + 1 dilutions were made, using the rest of the low insulin serum as diluent. Thus the following standards were obtained 206, 106, 56, 31, 18.5, 12.3, 9.2, and 6 μU/ml. In all cases the identity of the samples was withheld from the participants.

Results

First group experiment

The individual group modifications of the insulin RIA technique are shown in Table 1. Because all unknowns were measured in one assay-run, the within assay coefficient of variation could be obtained from the 24 samples of the serum pool. This ranged between 3.4% and 14%. The scatter of the means of the 24 values from each laboratory was between 21 μU/ml and 46 μU insulin/ml and had a coefficient of variation of 21%. The results from the OGTT-sera had a similar scatter. The means of the measured insulin concentrations lay between 13 μU (fasting) and 82 μU/ml (60 minutes after glucose), and the coefficient of variation of 18% calculated in the lower concentration range increased to 33% in the higher concentration range. The result from the first group experiment was, that there was no improvement of inter-laboratory precision, when the same insulin standards were used by all groups.

Second group experiment

Because of the different methods for evaluating RIA data (logit- or parabola-transformation, graphic-manual procedures), which intrinsically yield different hormone concentration values, particularly at either end of the standard curve, all results were handled as raw counts in this second group experiment in contrast

*Supported by the Deutsche Forschungsgemeinschaft (SFB 51, München, and SFB 87, Ulm).

Received: 15 Feb. 1974 Accepted: 12 Mar. 1974

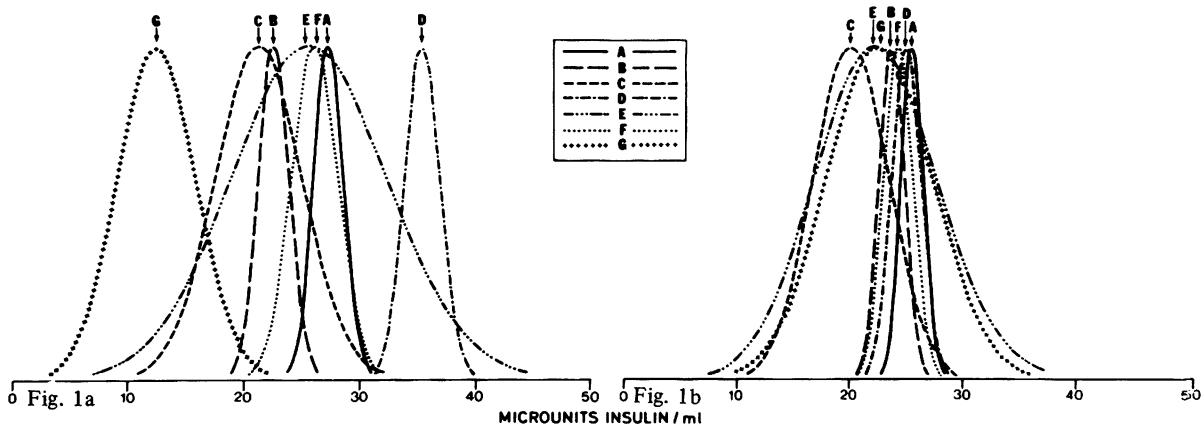

Fig. 1a Results of the insulin-determinations of the serum pool (n=9) as calculated from each laboratory's own standard curve in buffer (second group experiment). The results from each laboratory are represented in a Gaussian-curve. As usual the peak of each curve represents the mean value, the width of each curve between upper and middle third is two standard deviations. The peaks (means) have a scatter with a coefficient of variation of 28%. The results of the first group experiment (cf. text) were similar.
Fig. 1b The same data, related to the recovery curve, i.e. standards dissolved in low insulin serum. Coefficient of variation of the means 8%.

to the first one. In this way we were able to evaluate all hormone concentrations using one method, and so record only the differences of the assay variations. This was done with a computer program, using spline approximation to fit the standard curve. A Siemens 404/3 data processing machine was employed. The curves were plotted by an on-line controlled Hagen-plotter (*Marschner, Erhardt and Scriba* 1973). The count rates of the unknowns from the various groups were related firstly to their corresponding standard curve and then to their recovery curve.

Calculation of the unknowns, using the standard curve in buffer

The means of the 9 serum pool samples from all seven groups had a scatter with a coefficient of variation of 28%. This, and also the individual within assay precision were not appreciably different from the results of the first experiment (Fig. 1a). Similar results were also obtained by comparing the hormone values from the OGTT in both group experiments (Fig. 2a).

Calculation of the unknowns, using the recovery curve

By calculating the insulin concentrations of the samples from the serum pool (Fig. 1b) and those from the OGTT (Fig. 2b) using the recovery curve (see above) we obtained a great improvement of inter-laboratory precision. Inspite of the fact, that the individual within-assay precision remained nearly uninfluenced, the scatter of the means of the pooled serum was reduced to a coefficient of variation of only 8% from 28%.

It was noticed, that the recovery curves in serum generally had a gradient steeper than the standard curves in buffer (Fig. 3).

Table 1 Modifications of the radioimmunological determinations used by the participants.

	Serum Volume µl	Pre-Incubation h	Incubation h	Temp. °C	Pipetting Steps	B/F-Separation
A	100	6	24	4	3	Filtration on Cellulose Acetate Filters,
B	100	6	18	4	3	Using Preprecipitated Antibody
C	100	–	17-24	4-8	2	Solid-Phase Technique Fa. Boehringer
D	100	–	72	4	4	Ion Exchange Resin (Amberlite)
E	100	–	24	22	6	Sepharose-bound Antibody Pharmacia, Uppsala
F	50	3	8	22	3	Sephadex G-75-Column Chromatography
G	100	–	120	37	3	Dextran Coated Charcoal Adsorption

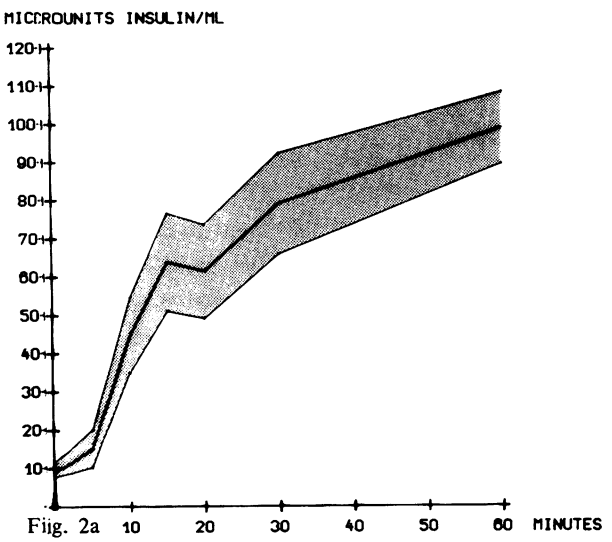

Fig. 2a

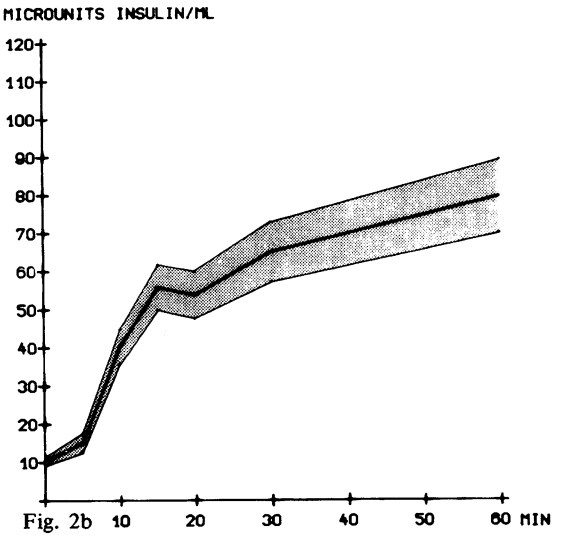

Fig. 2b

Fig.. 2a Results of the insulin-determinations in the sera from the OGTT, related to each laboratory's own standard curve in buffer. Ordinate: insulin concentration in μU/ml. Abscissa: time of venipuncture after glucose administration. The middle line connects the means of all single values of all laboratories, the shaded area covers two standard deviations. Mean coefficient of variation 14%.

Fig.. 2b The same data, related to the recovery curves: mean coefficient of variation 6.5%. In the higher concentration range of the standard curve no improvement was obtained (decreasing steepness).

We examined the usefulness of various human fasting sera for insulin recovery curves and prepared 7 recovery curves as described above. Triplicates of 4 control samples calculated by the computer on each of the 7 recovery curves, showed a coefficient of variation not greater than 7%, which lays within the precision of the assay.

Discussion

Group experiments are a well accepted procedure to examine the validity of laboratory results. For ordinary laboratory methods one or two determinations of a substance are sufficient for such a project whereas because of the nonlinear RIA standard curves 6 to 8 determinations in triplicates of samples having concentrations which are spread over the sensitive part of the standard curve are essential.

It is desirable, that the concentrations of the samples are within a physiological context, like in our case from an OGTT.

To make sure, that the results represent all individual laboratory errors, each participant should handle his samples as in a routine assay. A detailed information about the individual assay is not necessary. The only important fact is the agreement of results with those of other laboratories.

Since one is commonly interested in comparing assay systems and not the different evaluation procedures, it is advantageous to ask for count rates instead of hormone concentrations. From the obtained count rates the hormone concentrations will be

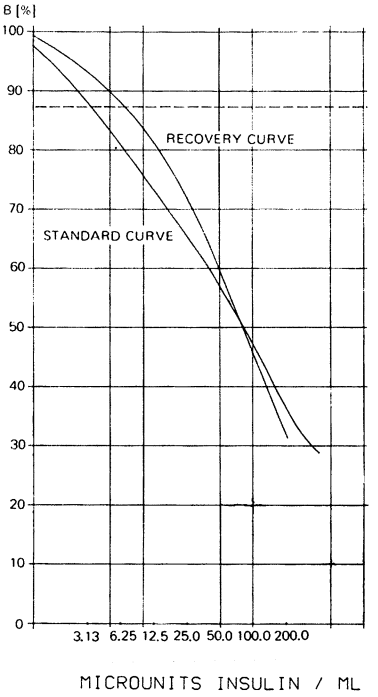

Fig. 3 A typical standard curve in buffer and recovery curve. The latter has a steeper gradient. Ordinate: B/B_0 (B = antibody bound activity, B_0 = maximal antibody-bound activity in absence of cold hormone). Abscissa: logarithmic dose (μU insulin/ml). The horizontal line at 87% is the limit of sensitivity, calculated as 3 standard deviations of B_0-values (n = 9).

calculated in a uniform fashion by an appropriate computer program. From the count rates of the standard curves one gets also information about the steepness of the slope of the standard curves and the limits of sensitivity (threefold standard deviation of the zero-standard).

The two group experiments, regarded as pilot studies, show, that both the type of the RIA system (antibody, bound-free-separation, incubation-time and -temperature, sample volume) are of secondary importance in improving inter-laboratory precision. It is, therefore, less important to standardize either of these. Differences in the purity of the tracer, the incubation and the antigen-antibody-reaction seem to play a greater role in serum samples than in pure buffer systems. In view of this, it was considered better to dissolve the insulin standard not in buffer, but in serum with a low basal insulin concentration. Comparable effects have been shown by *Erhardt, Marschner, Pickardt, and Scriba* (1973) in the measurement of hTSH.

A coefficient of variation greater than 20% is considered too high for satisfactory hormone measurement, particularly when needed frequently, for clinical and research purposes and when the RIA itself is relatively simple (*Ashford* et al. 1969). This can be improved considerably, however, by using standards in serum. In this way the inter-laboratory precision approximates to the range of such assays as cholesterol or urea-nitrogen (*Stamm and Büttner* 1969).

It is apparent, that the precision will decrease a little, if each laboratory uses a different serum for its own standards. Despite this, our results suggest, that this decrease would not be great. It is, furthermore recommended that standards be made by serial dilutions of 1 + 1, starting from one stock solution containing about 200 to 300 μU/ml. Thereby, it is necessary to use only one pipette and one single volume, resulting in a precision superior to all other ways of pipetting standard dilutions.

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