

The Relationship between Endogenous Serum Insulin Concentration and Glucose Uptake in the Forearm Muscles of Nondiabetics

NIELS JUEL CHRISTENSEN and HANS ØRSKOV

From the Second Clinic of Internal Medicine, Kommunehospitalet, Århus University School of Medicine, Århus, Denmark

A B S T R A C T In a series of experiments on the human forearm, preparation designed to examine the effects of variations in immunologically determined endogenous serum insulin levels and of blood glucose concentrations on muscular glucose uptake, the following results were obtained: (a) A highly significant correlation between muscular glucose uptake and simultaneous arterial serum insulin concentration. (b) No correlation between glucose uptake and simultaneous arterial blood glucose concentration during hyperglycemia. (c) A maximal insulin effect on muscular glucose uptake at arterial serum insulin concentrations at about $200 \mu\text{U}/\text{ml}$. This observation is, however, based on only a few experiments.

INTRODUCTION

During forearm studies designed to evaluate peripheral utilization of glucose, a linear relationship between endogenous insulin concentration and muscular glucose uptake was observed (1). The present investigation was undertaken in order to study this relationship in more detail and to see to what extent it was influenced by the blood glucose concentrations.

It is well known from *in vitro*, and it has been postulated on the basis of *in vivo*, experiments, that muscular glucose uptake is influenced by both the glucose and the insulin concentration of the buffer medium, perfusate, or plasma.

The present investigation suggests that plasma insulin concentration is the major factor in the

regulation of glucose uptake in the intact human forearm muscle, and that this preparation is not directly influenced by the blood glucose concentration.

METHODS

The glucose concentration of the whole blood samples was determined with a glucose oxidase method. A detailed report on the method used in our laboratory has been published elsewhere (2). Serum insulin concentrations were measured according to Hales and Randle (3) with ethylenediaminetetraacetate (EDTA) addition. This double antibody technique was checked carefully by comparison with a chromatographic procedure to ensure that no "unspecific plasma inhibitors" would interfere. A rapid and very reliable chromatimmunoassay, developed by one of us (4) was used for this purpose. With both methods, values are identical and serum insulin concentrations in 10-hr fasting, nonobese, normal adults averaged 20.2 ± 6.3 (mean \pm SD) $\mu\text{U}/\text{ml}$. Blood flow was estimated by the classical venous occlusion technique with a water plethysmograph thermostatically controlled at 34°C (5). Room temperature was controlled at $21^\circ\text{C} \pm 1^\circ$ (mean \pm SD).

11 medical students weighing 90–110% of ideal weight and between 20 to 30 yr of age were studied. Experiments were carried out in the morning after an 8–10 hr fast. A Teflon catheter was inserted into one brachial artery and another was inserted into a deep vein of the opposite arm.

The main difficulty in the experimental procedure was to make sure that *only* muscular blood is aspirated. To verify this, dye was injected into the superficial venous compartment before the experiment was started. Even in cases where palpation left no doubt that the catheter was placed deeply into the musculature, Evans blue dye, injected into the superficial vein, could sometimes be aspirated from the deep venous catheter, which indicated that the deep venous blood sample was contaminated with superficial venous blood. When repeated attempts failed

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to place the catheter so that no dye could be aspirated, the experiment was canceled.

Blood flow was measured in the forearm that contained the deep vein catheter before and after each blood sampling, and the mean flow was multiplied by the glucose A-V difference for the calculation of glucose uptake. After collection of fasting samples, an injection of 50%

glucose solution was given as a priming dose immediately before an intravenous glucose infusion was started in the arm not used for venous sampling.

We are well aware that the estimation of both forearm blood flow and of forearm volume does not represent muscle tissue solely. The resulting errors, however, tend to cancel out each other [for discussion, see (6)].

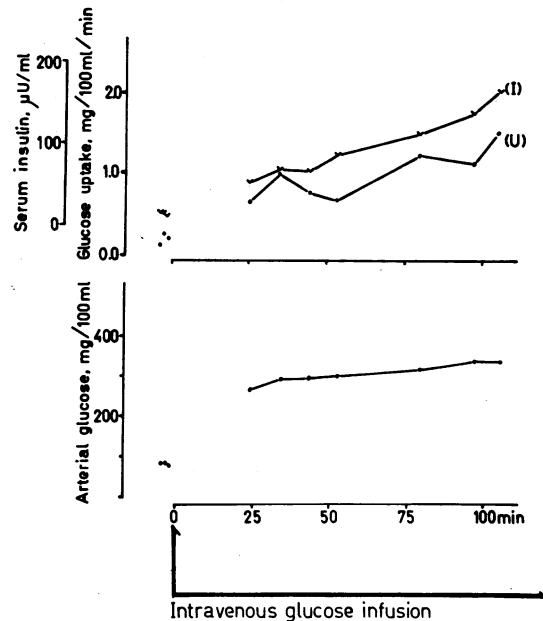


FIGURE 1 (case 4) Increasing hyperglycemia.

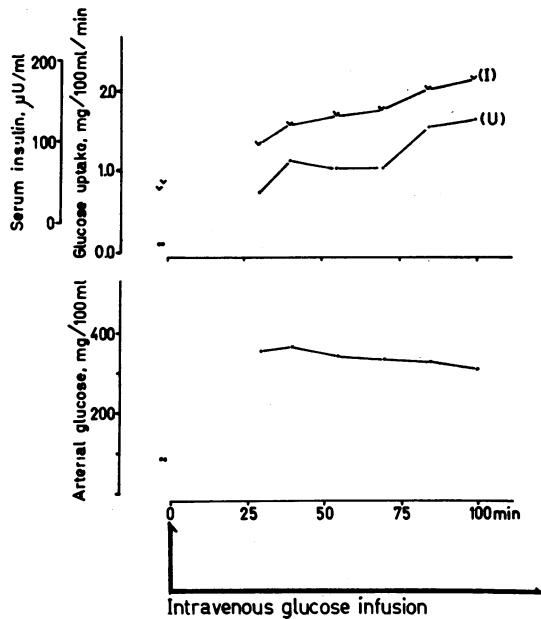


FIGURE 2 (case 7) Decreasing hyperglycemia.

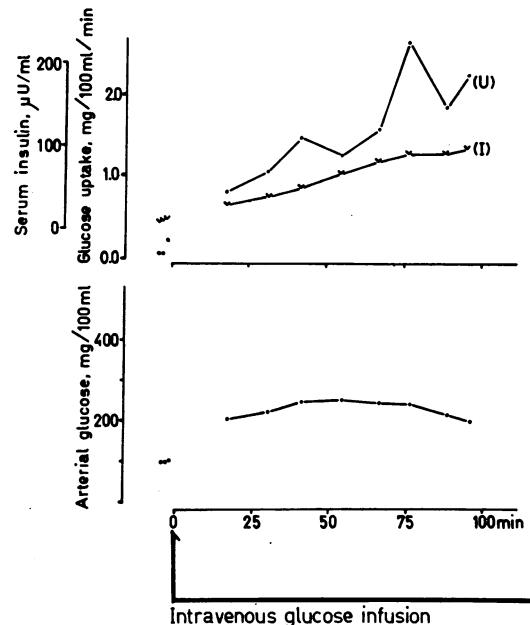


FIGURE 3 (case 10) Fairly constant hyperglycemia.

Arterial serum insulin (*I*), glucose uptake (*U*), and arterial glucose concentration (lower curve) in experiments representative of the three patterns of hyperglycemia.

The amount of glucose infused was varied to obtain either a constant level or a level of decreasing or increasing hyperglycemia. The rate of infusion depended on the blood sugar concentration obtained every 10 min during the experiment by a technique for rapid determination (7). In this way abrupt alterations in the desired pattern of hyperglycemia were avoided. The high blood glucose level was maintained for approximately 100 min. In order to ensure a relatively steady state, collection of samples for insulin and glucose determinations did not begin until 15–30 min after glucose infusion had started. It was not always possible to obtain sufficient amounts of deep venous blood for insulin determinations. During sample collections and flow determinations circulation to the hand was obstructed with a pressure cuff.

RESULTS

Fasting state. The mean arterial glucose concentration was 78 ± 5.4 mg/100 ml (mean \pm SD). The mean (\pm SD) fasting glucose uptake was 0.08 ± 0.05 mg/100 ml forearm per min. Of the 29 determinations in 11 subjects, only one was negative. The mean fasting glucose uptake differed significantly from zero (P less than 0.001). The mean (\pm SD) fasting serum insulin level was 28 ± 12 μ U/ml. In 18 measurements of the arteriovenous difference in seven subjects, nine were positive, two were zero, and seven were negative, i.e., we found no insulin uptake in the fasting state. The difference never exceeded 6μ U/ml.

During glucose loading. The blood glucose level rose to initially 170–350 mg/100 ml and, thereafter, followed three different patterns. In experiments 1–4 and 5 the glucose level showed a steady and slow increase of 1–2 mg/100 ml per min. In experiments 6–8 the glucose level decreased slowly and steadily 1–2 mg/100 ml per min. Finally, in experiments 9–11 the hyperglycemia remained unchanged throughout the loading period. Representative results from experiments showing each of the three different patterns of hyperglycemia are given in the lower parts of Figs. 1–3.

The serum insulin concentration of the first sample from the infusion period was increased in all cases. With the exception of experiment 6, serum insulin concentrations continued to rise during glucose loading, irrespective of whether blood glucose increased or decreased. In one experiment (case 6) insulin did not vary during hyperglycemia. The patterns of slowly increasing insulin concentrations in all 11 experiments are seen in Fig. 4. In the upper part of Figs. 1–3 the insulin re-

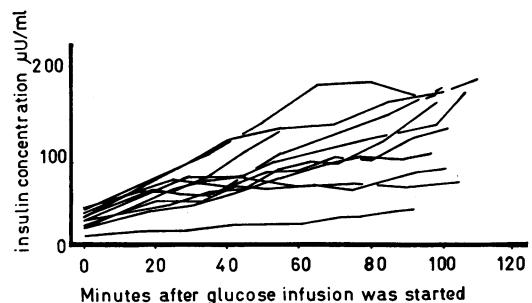


FIGURE 4 Changes in serum insulin concentrations during the 11 experiments.

sponses are shown together with arterial glucose concentrations and glucose uptakes.

Glucose uptake was elevated in the first sample from the infusion period in all cases. Thereafter it continued to rise, following the rise in serum insulin, irrespective of the blood glucose variations [see Figs. 1–3 (upper part)].

There was no tendency to systematic changes in forearm blood flow during the glucose loading. Table I records the mean blood flow of all experiments in samples 1, 2, etc. The sample to sample variations were not very large in the individual experiments. In two cases only, flow determinations from successive blood samples exceeded their mean by more than 20%. Finally there was no association between arterial glucose concentrations and forearm blood flow (P , larger than 0.3).

Pooled data, obtained during glucose infusion and including all observations from all subjects, showed no association between blood glucose concentrations and glucose uptake with the median test by chi square (P , greater than 0.1 for the absence of a positive correlation).

The same negative result was obtained by regression analysis, using serum insulin and blood glucose concentrations as invariables and glucose uptake as the variable (P , greater than 0.3 for the absence of a correlation between glucose concen-

TABLE I
Mean Value of Flow Determinations (ml/100 ml forearm per min) from Successive Samples during the Loading (all experiments).

Sample No.	1	2	3	4	5	6	7	8	9	10
Flow	3.2	3.2	3.2	3.2	3.4	3.1	3.3	3.4	3.1	2.4
n	11	11	11	11	11	9	6	5	4	1

n, number of subjects in whom samples 1–10 were obtained.

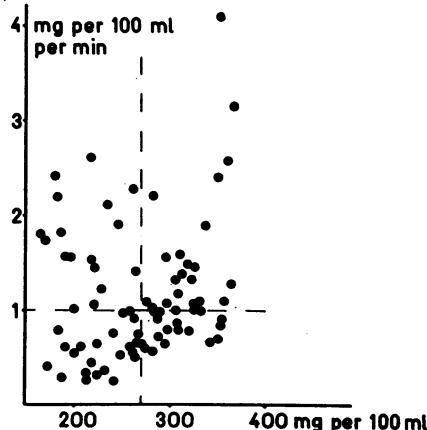


FIGURE 5 Pooled data of glucose uptake and arterial glucose concentration during glucose infusion. The stippled lines indicate the medians.

tration and uptake). However, a positive correlation was found by the median test between serum insulin and glucose uptake (P , less than 0.005). The results are illustrated in Figs. 5 and 6. Regression analysis was performed on all observations of serum insulin concentrations and glucose uptake in the individual experiments. When glucose uptake was plotted as a function of serum insulin concentrations, an obvious rectilinear relationship between the two parameters was observed. In all cases, regression analysis showed that the slope of the regression line differed highly significantly from zero. The results are summarized in Table II where the standard deviation from the regression line is also given. Fig. 7 shows the association obtained in all 11 experiments.

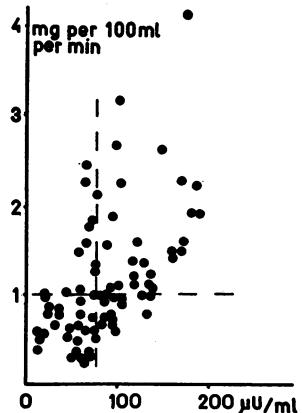


FIGURE 6 Pooled data of glucose uptake and arterial serum insulin concentration during glucose infusion. The stippled lines indicate the medians.

TABLE II
Statistical Analysis on Determinations of Glucose Uptake (y) and Serum Insulin (x) in the Individual Experiments.

Case No.	Equation of the regression line	P less than	No. of determinations	$\pm SD^*$
1	$y = 0.012x - 0.31$	0.001	12	0.27
2	$y = 0.024x + 0.07$	0.001	14	0.21
3	$y = 0.008x - 0.05$	0.01	11	0.15
4	$y = 0.009x + 0.03$	0.001	10	0.14
5	$y = 0.012x - 0.14$	0.01	8	0.27
6	$y = 0.044x - 1.18$	0.005	8	0.41
7	$y = 0.012x - 0.46$	0.001	8	0.11
8	$y = 0.014x - 0.47$	0.001	8	0.24
9	$y = 0.010x - 0.02$	0.001	12	0.21
10	$y = 0.024x - 0.22$	0.001	11	0.29
11	$y = 0.026x - 0.45$	0.01	7	0.82

The regression lines are shown in Fig. 7.

* Standard deviation about the regression lines.

In experiment 5 endogenous insulin concentrations exceeded 200 μ U/ml at three points of time. Above this latter level the linear relationship between glucose uptake and serum insulin levels did not hold, i.e., there was no further increase in glucose uptake. These three values were not included in the regression analysis. In two other cases, 2 U of human insulin¹ were given intravenously after

¹ NOVO Research Institute, Copenhagen.

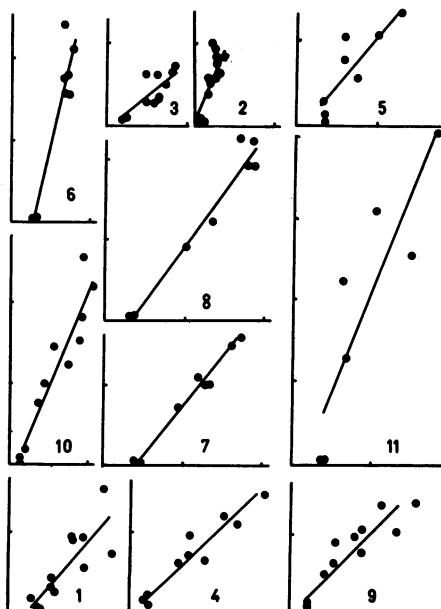


FIGURE 7 Regression lines and all determinations of serum insulin and glucose uptake in the individual experiments. 1 U on the abscissa = 100 μ U/ml. 1 U on the ordinate = 1 mg/100 ml per min.

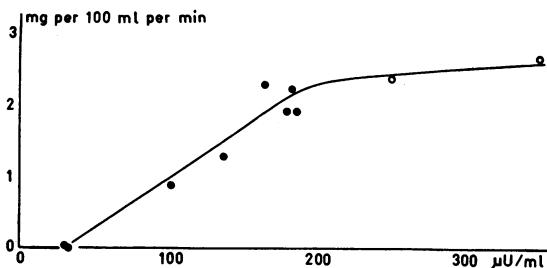


FIGURE 8 Relationship between serum insulin and glucose uptake in an experiment where serum insulin exceeded 200 $\mu\text{U}/\text{ml}$ (open circles).

the experiment. After 15 min, collection of blood samples was resumed. Glucose uptake increased in neither case (Fig. 8). In fact, the glucose uptakes corresponding to the highest arterial insulin concentrations in each of the three experiments (mean = 286 $\mu\text{U}/\text{ml}$) averaged 106 per cent of the glucose uptakes which corresponded to the lowest insulin concentrations obviously outside the linear part of the relationship curves (mean = 224 $\mu\text{U}/\text{ml}$). It should be noted that the blood glucose level declined in none of these experiments.

Statistical analysis shows that, if the regression lines calculated from arterial insulin concentrations lower than 200 $\mu\text{U}/\text{ml}$ (Table II) are extrapolated to high levels of plasma insulin, the glucose uptake values observed in seven samples with high insulin concentrations are significantly below the extrapolated lines (average = 55%; P , less than 0.001). If the same calculation is performed on the assumption that glucose uptake is a function of log-insulin concentration, the glucose uptake values are also too low (average = 76%; P , less than 0.05).

DISCUSSION

The experiments reported in the present paper were designed to investigate the sensitivity of muscle to insulin in man, i.e., the relationship between endogenous plasma insulin concentrations and glucose uptake, using the forearm for estimating muscular glucose uptake.

The forearm preparation has been employed in extensive studies of peripheral muscular glucose uptake by Butterfield, Fry, Holling, Garratt, and Whichelow (8-11) and by Zierler and Rabinowitz (12).

Butterfield et al. and Butterfield, Abrams, Sells,

Sterky, and Whichelow (8, 9, 13) arrived at the conclusion that arterial blood sugar must exceed a certain threshold value before glucose will enter the muscle cells, and that the effect of insulin is to lower this threshold, whereas insulin apparently does not alter the relationship between rise of blood glucose and increase in glucose uptake. However, in their experiments on nondiabetics where they used an intravenous glucose infusion to study the relation between arterial glucose concentrations and muscular glucose uptake, the results they observed with increasing blood sugar values may just as well have been caused by a concomitant rise in plasma insulin. On the other hand, Andres and Zierler (14) reported in abstract form that intra-arterially infused glucose did not stimulate glucose uptake. Their experiments were performed in fasting subjects. In other investigations Butterfield and his associates (10) studied the effect of radioactive beef insulin injected intra-arterially. They demonstrated a correlation between fixed amounts of radioactivity and muscular glucose uptake, and between fixed amounts of radioactivity and plasma insulin concentration. These relationships were highly significant and suggested a positive correlation between insulin concentration and muscular glucose uptake. The results of their study, however, are not easy to interpret, partly because the experiments were carried out at extremely high plasma concentrations of radioactive insulin and partly because of difficulties inherent in using a radioactive preparation for the estimation of relatively small degrees of insulin fixation, since such preparations always contain significant amounts of impurities.

Langs and Andres (15) reported briefly experiments which showed that glucose uptake in the human forearm increased when beef insulin was infused intra-arterially, in fasting subjects, at such a rate that plasma concentrations increased about 40 $\mu\text{U}/\text{ml}$. However, Zierler and Rabinowitz (16) could not confirm in fasting subjects that increments in arterial plasma insulin concentrations of 40 $\mu\text{U}/\text{ml}$ increased glucose uptake. In similar experiments Andres, Baltzan, Cader, and Zierler (17) demonstrated a definite increase in the glucose uptake when the plasma insulin concentration was increased by 200-700 $\mu\text{U}/\text{ml}$.

Zierler and Rabinowitz (12) discussed the results obtained by other authors who used isolated

tissue (rat diaphragm and heart), and emphasized the differences between *in vivo* and *in vitro* conditions. The most important differences are that the basal glucose uptake is 50 times higher in *in vitro* preparations and that the percentage-response to insulin is much lower.

In a situation much more comparable to the human forearm, the hind-limb preparation (18, 19) (resting muscle perfused with blood), the basal glucose uptake exceeds that of the forearm by a factor of four and the percentage-response to insulin is higher than in isolated tissue.

Rabinowitz, Klassen, and Zierler (20) have also shown that intra-arterial infusion of growth hormone decreases both the basal glucose uptake and the effect of simultaneously infused insulin.

Studies with *in vivo* and *in vitro* preparations thus seem to indicate that insulin as well as glucose exerts a stimulating effect on muscular glucose uptake. However, comparisons between these two types of preparations are difficult for the reasons stated above, and those studies in which a stimulating influence of glucose on the human forearm was shown do not exclude the possibility that this effect is secondary to an increase in plasma insulin. In regard to the effect of growth hormone, we do not know if the usually low concentrations with sudden increments, characteristic of plasma growth hormone after an overnight fast (21), influence the glucose uptake in the human forearm to any significant extent.

As yet, no one has tried to correlate glucose uptake to simultaneous endogenous insulin levels under circumstances where the influence of plasma growth hormone can be ruled out, i.e., equals zero. Samols, Marri, and Marks (22) were unable to find any relationship between immunological serum insulin concentrations and arteriovenous differences (not glucose uptake) under conditions where blood glucose showed rapid and large variations. In the present investigation a linear relationship between glucose uptake and immunologically determined serum insulin concentrations was demonstrated in nondiabetics under circumstances (prolonged hyperglycemia) where it is reasonable to believe that growth hormone was not present. On the other hand, there was no correlation between glucose concentrations and glucose uptake. It is not possible on the basis of these studies to rule out completely the possibility that the glucose

concentration per se influences glucose uptake. It is of course unreasonable to believe that the relationship between insulin level and glucose uptake obtains under all circumstances; for instance, under the pharmacological conditions of insulin-induced hypoglycemia. We suggest, however, that the insulin concentration of arterial blood is the primary factor responsible for the stimulation of glucose uptake in intact muscle, and that blood glucose has no direct effect. This suggestion is consistent with the fact that zero or exceedingly low glucose uptakes are found in the classical hyperglycemia of untreated juvenile diabetics, as well as during induced excessive hyperglycemia, since these patients have low fasting insulin levels and no insulin response to glucose loading. The suggestion also fits well with our observations in three untreated nonketotic juvenile diabetics, where we have found a definite response in glucose uptake to exogenous insulin.

These considerations, combined with the previously mentioned fact that increases in plasma growth hormone depress uptake, make it reasonable to assume that the extent to which glucose enters the resting muscle cells depends on the state of equilibrium existing between plasma insulin and growth hormone.

It would be of interest to study insulin sensitivity, i.e., serum insulin-glucose uptake relationships, in juvenile and maturity onset diabetics in different states of metabolic derangement during relatively constant hyperglycemia, such as was achieved in the experiment reported here.

Our finding that glucose uptake increases either less than was expected or not at all, when the serum insulin concentration exceeds about 200 μ U/ml; might mean that insulin stimulation is maximal at this level. The phenomenon is now being studied in greater detail. A serum insulin concentration in the vicinity of 200 μ U/ml corresponds to the upper physiological range that obtained in the course of the day. A similar flattening of the dose-response curves is known from *in vitro* biological assays of insulin.

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