Muscle Glucose Uptake Does Not Increase When Only Local Arterial Glucose Concentration Is Increased

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Published models of mammalian whole-body glucose metabolism generally assume that glucose uptake is proportional to circulating glucose concentration at constant insulin concentration. One widely used model labels the increased whole-body glucose uptake seen with increased venous glucose concentration as "glucose effectiveness." In 1956 and 1957, we found on average no change in forearm glucose uptake when we doubled, or more than doubled, local forearm arterial glucose concentration by close arterial infusion so that pancreatic arterial glucose concentration did not change. These experiments are being reported in extenso for the first time. Since that time, two other groups have found in glucose/insulin clamp experiments that whole-body glucose uptake was not proportional to hyperglycemic concentrations, although uptake did increase. It was hypothesized that perhaps some organs and tissues do increase uptake proportionally and other tissues not at all. Our results show that skeletal muscle is a tissue in which glucose uptake, at constant insulin, is not changed acutely by hyperglycemia; there is no forearm glucose effectiveness. We suppose that this constancy occurs because there are so few GLUTs on the sarcolemma surface in the basal state and that they are saturated even at euglycemia. We also report earlier experiments from 1954 to 1955 in which comparable hyperglycemia was reached by a large oral dose of glucose, with a 10-fold increase in glucose uptake. The arterial glucose time curve during hyperglycemia was 20–30 mg/dl higher than the forearm venous curve. ***Diabetes*** **51:2698–2702, 2002**

Since the pioneering work of Steele and colleagues (1,2), the dominant models of in vivo glucose uptake in mammals have assumed that at constant insulin concentration, glucose uptake is proportional to the concentration of glucose in blood or plasma. This assumption is retained in the current widely used minimal model of Bergman et al. (3). This model includes a pair of coefficients to relate glucose uptake to blood glucose concentration. One of these coefficients depends on insulin concentration and is therefore constant at constant insulin concentration, and the other is a constant and is therefore a measure of what Bergman et al. have labeled "glucose effectiveness," defined as the "influence of glucose at basal insulin to enhance its own utilization and suppress its own endogenous production" (4).

In 1958 we reported, at an annual meeting of the Federation of American Societies of Experimental Biology, that when glucose was infused into a brachial artery at constant rate in order to increase blood glucose concentration supplying forearm tissues without elevating recirculating blood glucose concentration, there was, on average, no change in glucose uptake (5). We never submitted a manuscript describing these experiments for definitive publication in extenso. Consequently, the observations had no influence, and the assumption that glucose uptake is proportional to blood glucose concentration persists. Best, a coauthor of the 1996 report on "glucose effectiveness," had an earlier report titled "Glucose disposal is not proportional to plasma glucose level in man" (6), a conclusion based on glucose and insulin clamp studies. Verdonk et al. (7), independently and nearly simultaneously, designed and carried out quite similar experiments, with similar results. Whole-body glucose uptake did increase with increased glucose concentration, but at a decreasing proportion. Best et al. (6) hypothesized that some organs and tissues did increase glucose uptake proportional to glucose concentration, but other organs and tissues had a fixed glucose uptake. The hypothesis that there may be some organs and tissues for which glucose uptake is fixed over some range of blood glucose concentrations has only been tested (before the hypothesis was formulated) in our unpublished experiments on forearm glucose uptake, carried out in 1956–1957. This is a report of those experiments, in which we demonstrated that forearm muscle glucose uptake is on average constant over the range of brachial arterial glucose concentrations, from normal to a little more than twice normal. In addition, the results of these experiments, in which there was hyperglycemia in the local blood supply to the forearm only and hence no stimulus to the pancreas to release insulin, are compared with those in which oral administration of glucose caused hyperglycemia throughout the circulatory system, therefore stimulating insulin release and causing increased forearm glucose uptake.

RESEARCH DESIGN AND METHODS

A total of 10 healthy young men (mean age 28.3 years, range 21–36) were involved in our studies of metabolism of the forearm during 1954 through

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A-V, arteriovenous.

1957. Nine were either medical students or young physicians (house officers or postdoctoral fellows). One was a hospitalized patient convalescing from lobar pneumonia. He had been admitted to the hospital 9 days before, and penicillin had been discontinued 48 h before the study. He was ambulatory and awaiting discharge. We obtained self-reported height and weight on only three subjects. Their BMIs were 21.4, 21.9, and 22.5 kg/m^2. No one appeared obese or undernourished. The procedure was as previously described (8). Briefly, the subjects had nothing by mouth after the evening meal, presented themselves to the laboratory in the morning, and were prepared for the experiment. A double-lumen steel needle was inserted into a brachial artery. The downstream lumen was used for infusion at a constant rate of T1824 Evans' Blue Dye for measurement of forearm blood flow and for infusion of any other substance of interest; in this case, in some experiments, it was glucose. The upstream lumen was used for sampling arterial blood before it was enriched by the infusate. A catheter was threaded into an ipsilateral deep antecubital vein (the black vein of Holling), draining mostly muscle, and a catheter was threaded into an ipselateral superficial vein, draining skin and subcutaneous tissue. During infusion periods, a sphygmomanometer cuff was placed around the wrist and inflated above systolic pressure to occlude blood flow to the hand, so as to maximize arteriovenous (A-V) concentration differences across the forearm, which would otherwise have been diluted by the largely thermal blood flow to the hand. Wrist occlusion was never maintained for >40 min. Occlusion was reapplied after sufficient time for reactive hyperemia to subside.

In six subjects, studied in 1956 and 1957, after a control period in which the infusate was only Blue Dye in normal saline, the infusate was switched to Blue Dye in normal saline plus glucose at a final concentration (always measured exactly) of ~60 mg/ml (333 mmols/l), delivered at a rate (measured exactly) of ~0.1 ml/min or ~30 μmol glucose/min. At a brachial arterial flow rate of ~5 ml/min (measured in each subject by dye dilution), the infusate added ~6 mmol/l to incoming brachial arterial glucose concentration in the steady state. Glucose infusion was maintained for 36 min.

Four subjects studied in 1954 and 1955, instead of receiving intra-arterial glucose infusion, drank a solution of glucose. In three of these subjects, the glucose dose was 100 g in 500 ml distilled water, and in the fourth it was 63 g (1 g/kg body wt). Forearm arterial and venous glucose concentrations were measured at intervals for at least 150 min after ingestion.

Forearm blood flow was measured by dye dilution (8,9). For the subjects who received glucose intra-arterially, glucose concentration was measured in whole blood by a modified anthrone method (10). For the subjects who received glucose by mouth in the earlier experiments, blood glucose was measured by Nelson's adaptation of the Somogyi method (11). It was not until a few months after the last of these experiments was completed that our laboratory switched to the glucose oxidase method, which was new at the time. We compared basal resting blood glucose concentrations of our 10 subjects with the values obtained in 23 subjects by the glucose oxidase method. In the 23 glucose oxidase determinations, the mean basal glucose in arterial blood was 4.88 ± 0.27 mmol/l; in the four subjects for whom arterial blood glucose was determined by the Nelson-Somogyi method, the mean was 4.70 ± 0.08 mmol/l; and in the six subjects in whom blood glucose was determined by the anthrone method, the mean was 5.15 ± 0.39 mmol/l. The concentration of glucose in downstream arterial blood bathing forearm tissues during intra-arterial glucose infusion (local forearm arterial glucose concentration) was calculated as follows: brachial artery glucose concentration upstream from the site of infusion was measured by the anthrone method. The concentration of glucose provided by the infusate was added to this concentration, calculated as follows: the concentration of glucose in the aqueous solution of Evans' Blue Dye was measured as was the volume rate of injection, from which was determined the rate at which glucose was injected into the brachial artery. Blood flow was determined by the Blue Dye indicator-dilution method. The added glucose concentration in local forearm arterial blood was calculated from the rate of glucose injection (moles per minute) divided by forearm blood flow (milliliters per minute). This calculation is an accurate measure of glucose in an aqueous solution in which the only reactant is glucose. Blood glucose values calculated from the anthrone method were ~5% higher than those from glucose oxidase, but the calculation of added local glucose is not. This means that the unadjusted calculation of local arterial glucose by the anthrone method is <5% higher than true glucose, but the calculation of glucose in forearm venous blood is 5% too high; thus, the calculated A-V difference would be falsely low. To adjust for this methodological error, we have corrected blood glucose estimations by the anthrone method by a factor of 1/1.05. This correction has no effect on any qualitative conclusion in this report.

Glucose uptake was calculated as the product of blood flow and A-V glucose concentration difference.

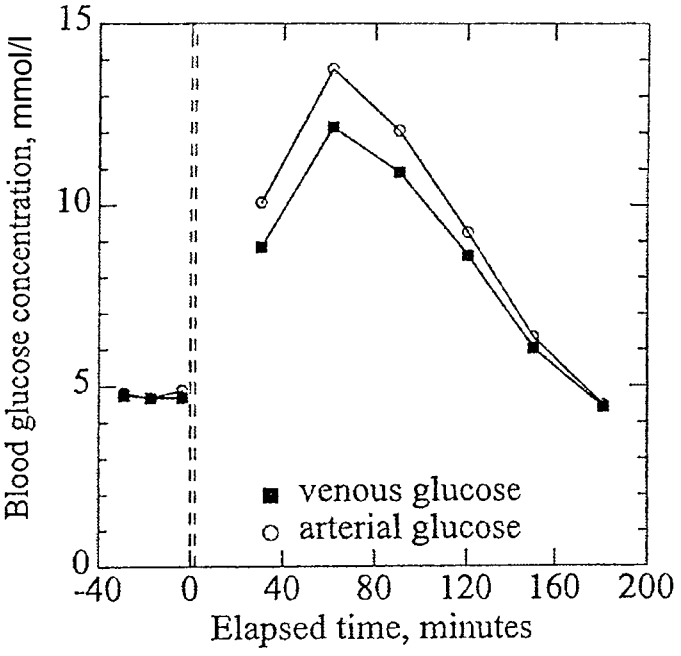

FIG. 1. Forearm arterial and venous glucose difference in response to a large oral dose, illustrated by values for one of the subjects. Vertical dashed lines indicate onset and completion of drinking the glucose dose.

RESULTS

Figure 1 illustrates arterial and venous blood glucose concentrations over time in response to the oral glucose load. In every subject, A-V difference over the period of the test was substantially higher than before glucose loading. Local brachial arterial glucose concentration peaked at ~60 min after the onset of the oral load. Simultaneous A-V concentration differences during rapidly changing arterial concentration are not accurate measures of cell uptake; they give falsely high results during rising arterial curves and falsely low results during falling, but, if the distribution of blood flow is stationary during the period between steady glucose concentrations, the integrated A-V difference during the interval between steady states, multiplied by blood flow, is a correct measure of total uptake during that period (12). Forearm blood flow during the period of the three highest blood glucose concentrations averaged ~90% of flow during the control period (73, 87, 96, and 100% in the four subjects). Therefore, the integrated A-V glucose concentration difference during transients from baseline to return to baseline multiplied by blood flow during that period could be a reasonably reliable measure of forearm glucose uptake over the duration of the transient. However, because we sampled blood glucose concentrations only at 15- or 30-min intervals, we did not have confidence in the accuracy of estimates of the integral, Therefore we restricted our calculations to three A-V differences at the peak and one on each side of the peak, where the absolute value of slopes of the glucose concentration time curve were minimum. Standard deviation for the four subjects was 1, 3, 13, and 15% of the mean of arterial glucose concentration measured at the three highest points, which we now designate relative plateaus.

During the period of hyperglycemia after the test dose of glucose, arterial concentration exceeded venous by a mean of 1.15 mmol/l (~20 mg/dl).

TABLE 1
Effect of oral glucose load on arterial glucose concentrations and forearm glucose uptake

	Arterial glucose concentration (mmol/l)		Forearm glucose uptake (μmol · min^{-1} · [100 ml forearm volume]$^{-1}$)	
Subject	Basal	30–90 min postglucose	Basal	30–90 min postglucose
1	4.60	13.10	0.68 ± 0.60	10.32 ± 1.50
2	4.72	10.16	0.41 ± 0.00	7.88 ± 1.07
3	4.80	10.76	0.38 ± 0.38	2.98 ± 0.62
4	4.67	9.69	1.41 ± 0.65	5.86 ± 2.28

Data are means ± SD. Oral glucose dose was 100 g in water (three subjects) or 1 g/kg body wt. Glucose concentration after oral glucose load is the mean of the three highest arterial concentrations, ~30, 60, and 90 min after the dose. Forearm glucose uptake was determined by the product of brachial arterial blood flow and A-V concentration differences on blood samples drawn simultaneously.

In all four subjects, the relative plateau arterial glucose concentrations were at least double those before the glucose load (Table 1). During that relative plateau period, glucose uptake increased by an average factor of 11.6 (range 4.2–19.2).

Figure 2 illustrates a response of arterial and venous glucose concentrations to intra-arterial glucose infusion. Venous concentrations do not reach the steady-state response to the new constant arterial concentration until a short time after the onset of the infusion; early A-V differences overestimate glucose uptake. Therefore, we used data only after the first 10 min of glucose infusion for calculation of effect, or lack thereof, of arterial glucose concentration on glucose uptake. Over that period, local forearm glucose A-V concentration difference was no greater than during the control period. Upstream brachial

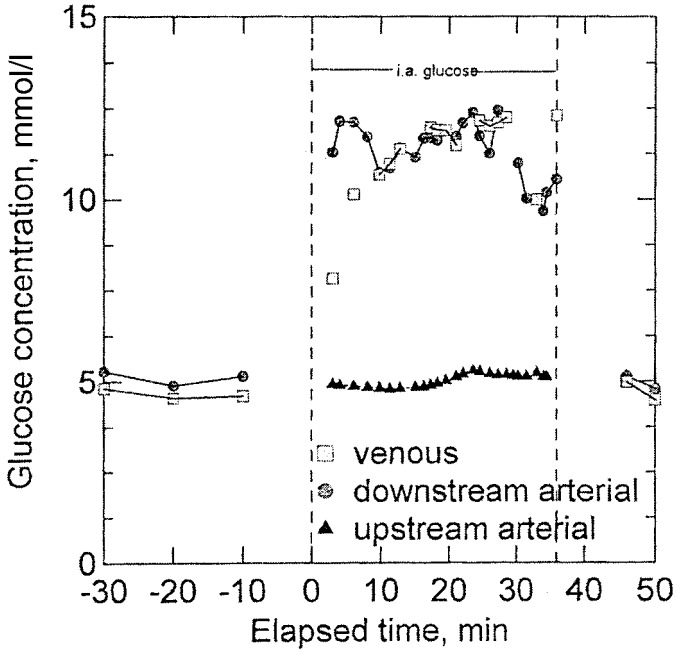

FIG. 2. Forearm upstream and downstream arterial glucose concentrations and forearm venous glucose concentrations, illustrated for one subject. Upstream and downstream arterial glucose concentrations are defined in Table 2 legend.

TABLE 2
Effect of local intra-arterial glucose infusion on arterial glucose concentrations and forearm glucose uptake

	Arterial glucose concentration (mmol/l)			Forearm glucose uptake (μmol · min^{-1} · [100 ml forearm volume]$^{-1}$)	
		During infusion (10–26 min)			
Subject	Basal	Upstream	Downstream	Basal	During infusion
5	5.11	5.04	11.34	1.63 ± 0.69	−3.51 ± 3.35
6	5.36	5.21	12.49	−0.95 ± 1.02	0.03 ± 2.90
7	4.70	4.65	10.06	1.28 ± 1.00	4.91 ± 3.00
8	5.68	5.23	9.02	0.95 ± 0.41	1.45 ± 3.45
9	4.66	4.70	14.50	0.54 ± 0.00	2.11 ± 1.12
10	4.84	4.87	12.58	0.85 ± 0.94	−0.98 ± 1.64

Data are means ± SD. Arterial glucose infusion was at a constant rate for 36 min. Because time is required for volume distribution of the added glucose to reach plateau concentrations downstream from site of injection, only data obtained during the last 26 min of infusion were used in computing glucose uptake during intra-arterial glucose infusion. Upstream glucose concentrations are those upstream from the glucose infusion site; they are glucose concentrations in the general systemic arterial circulation. Downstream concentrations during intra-arterial infusion are those distal to the infusion site, and each is the sum of an upstream concentration, with the glucose concentration added to brachial aterial blood by constant intra-arterial infusion.

arterial glucose concentration was the same as during the control period; therefore, the β-cell was not bathed by hyperglycemic blood.

Table 2 gives data for the six subjects who received a constant infusion of glucose. Resting blood glucose concentrations were not significantly different from those of the group receiving oral glucose, and brachial arterial glucose concentrations upstream from the infusion site were the same as those during the control period. Arterial glucose concentration increased to a group mean of 11.6 mmol/l, about the same as the increase in the oral glucose group. Glucose uptake during the control period was similar to that during the control period of those who received glucose by mouth. Glucose uptake during the steady-state period of intra-arterial glucose infusion varied widely, although mean glucose uptake by the group did not differ from control before glucose was administered intra-arterially. For the intra-arterial group, there were a total of 15 measurements during the control period. In five of the subjects, all 12 control glucose uptakes were positive. In a sixth subject, all three control uptakes were negative. During the steady-state intra-arterial period, there were 37 measurements, 10 of which were negative. All negative values for glucose uptake occurred in three of the subjects. Mean glucose uptake by the six subjects was the same during the infusion period (0.66 ± 1.7 μmol · min^{-1} · 100 ml forearm volume^{-1}) as it was during the control period (0.72 ± 0.32).

Figure 3 summarizes the data of Tables 1 and 2, displaying in the left frame the group mean (+SE) values of brachial arterial glucose concentration in the four subjects who had glucose by mouth and the six who received glucose via continuous infusion into the brachial artery. Control forearm arterial glucose concentration was not significantly different in the two groups. The increased

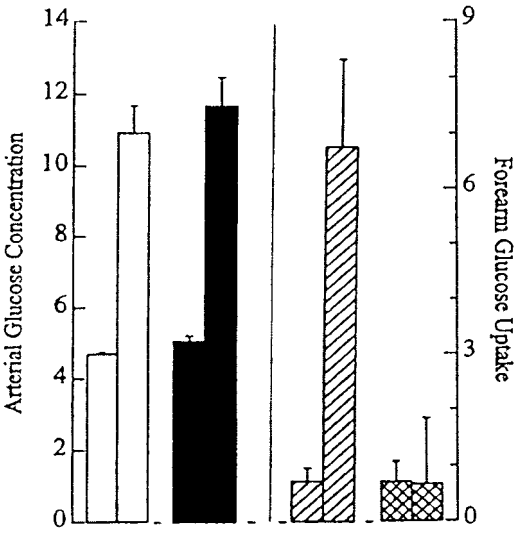

FIG. 3. Comparison of brachial arterial glucose concentrations and forearm glucose uptake between those given an oral load of glucose and those who had brachial arterial glucose infusion. The two pairs of bars in the left frame represent means ± SE of glucose concentration (mmol/l). The two pairs in the right frame represent means ± SE of forearm glucose uptake (mmol · ml^{-1} · [100 ml forearm volume]$^{-1}$). The left bar of each pair gives the value during basal euglycemia, and the right bar gives the the value during brachial artery hyperglycemia. The white and hatched bars are mean values of those given the oral glucose lead, and the black and cross-hatched bars are mean values of those given arterial glucose infusion.

local forearm arterial glucose concentraion produced by oral or by intra-arterial administration of glucose was also not significantly different in the two groups (both ~2.3 times the resting concentration). The right frame of Fig. 3 is a bar graph of forearm glucose uptake, shown as means ± SE. Mean control forearm glucose uptake was the same in the two groups. Mean forearm glucose uptake increased by a factor of nine in those who had glucose by mouth, but it did not change for those who were administered glucose intra-arterially. The difference between mean glucose uptake for those who drank the glucose solution (6.7 μmol · min^{-1} · 100 ml forearm volume^{-1}) and for those who were given glucose intra-arterially (0.66 μmol · min^{-1} · 100 ml forearm volume^{-1}) was 6.1 ± 1.94 μmol · min^{-1} · 100 ml forearm volume^{-1} (t = 3.13 and P = 0.005, approximately).

DISCUSSION

The central question we addressed is: does an increase in arterial glucose concentration, accomplished without stimulating insulin release into the circulation, cause an increase in forearm tissue (mostly muscle) glucose uptake? Before we consider it, we have to investigate the reason for negative numerical values of forearm glucose uptake, which seemed to occur more frequently when there was local forearm hyperglycemia in the group receiving intra-arterial glucose. Glucose uptake is the product of A-V difference and blood flow. Because blood flow can not be negative, the calculated negative value of glucose uptake must be due to negative A-V difference. That skeletal muscle lacks glucose-6-phosphatase and cannot produce and release glucose into venous blood draining muscle is well established. In our first full publication of studies of forearm, metabolism in 4 of 14 normal subjects' forearm glucose A-V differences was negative (8). In the present study of effects of intra-arterial glucose, glucose A-V concentration difference was 0.24 mmol/l (range of means of the six subjects −0.17 to 0.45) during the control period and 0.14 mmol/l (−1.3 to 1.3) during the steady-state intra-arterial infusion. The A-V glucose concentration difference was ~5% of arterial glucose concentration during the control period and slightly >1% during the steady-state intra-arterial infusion. We reported that standard deviation of triplicate blood glucose measurement by the anthrone method is 0.079 mmol/l, or ~1.6% of normal arterial glucose concentration (8). Given analytical error of reproducing replicate values for glucose concentration, cumulative analytical errors are adequate to produce occasional negative values for A-V glucose concentration difference. Note that in the mid-1960s, when our lab was using a specific glucose oxidase method, we found, in 23 normal healthy subjects, that brachial arterial glucose concentration was 4.90 mmol/l and forearm A-V glucose difference was 0.28 mmol/l (range 0.07–0.51); no negative A-V differences were found.

When we first made the observations reported here, GLUTs had not been identified; the story of recruitment of GLUTs from the cell interior to the plasma membrane had not been proposed, let alone elucidated, and it was not known that the predominant GLUT in sarcolemma of resting muscle in the basal state is GLUT1, that there is relatively little of it in the basal state, and that, in skeletal muscle, insulin recruits predominantly or only GLUT4 (see 13 for review and references). Ingesting 100 g (or even 63 g) of glucose raises the systemic arterial glucose concentration, stimulates release of insulin, increases the amount of GLUT4 in muscle sarcolemma, and may also increase the efficiency with which GLUT4 and perhaps also GLUT1 transport glucose (14), therefore accounting for the 10-fold increase in forearm muscle glucose uptake under these conditions. On the other hand, because we did not permit pancreatic arterial glucose concentration to rise when we increased local brachial arterial glucose concentration to the same degree of increase with an oral glucose load, the local brachial arterial glucose during intra-arterial glucose infusion supplied muscles in which the small number of sarcolemma surface GLUTs were operating under saturation conditions. In the absence of insulin or some other stimulus known to recruit GLUT4 to sarcolemma surface (e.g., hypoxia or exercise), there was, on average, no increase in forearm glucose uptake during doubling of local arterial glucose concentration. For resting skeletal muscle with the subject in a basal state, there was no evidence, under the conditions of these experiments, that increasing the local glucose concentration is by itself acutely able to cause increased glucose uptake, and there is no evidence in forearm skeletal muscle for the phenomenon that has been called "glucose-dependent glucose uptake." In the basal state, muscle glucose uptake, within the limited range of observation of the experiments reported here, is zero order, not first order. Skeletal muscle, under the conditions of our experiments, is at least one of the tissues whose existence was hypothesized by Best et al. (6) that, at constant insulin concentration, do not increase glucose uptake as glucose load increases. Our observations on constancy of forearm glucose uptake

despite increased local brachial arterial glucose concentration show that, contrary to the assumption of the "minimal model" (2), the rate at which glucose is transferred from blood into cells is not a constant proportion of blood glucose concentration at constant insulin concentration. Indeed, it was a poor assumption in theory because we know that transfer of glucose into cells is a saturable process and hence cannot be described as simply proportional to blood glucose concentration. Furthermore, the concept of "glucose effectiveness" (3) is invalid for the case of acute response by skeletal muscle because forearm glucose uptake was not increased by local hyperglycemia under circumstances in which there could be no increase in insulin concentration.

Our experiments with oral glucose were carried out several years before insulin radioimmunoassay was available. There can be no doubt that the oral dose of glucose raised systemic arterial glucose concentration high enough to stimulate insulin release from β-cells. We know from these experiments that when we gave enough glucose by mouth to double arterial glucose concentration, there was about a 10-fold increase in forearm glucose uptake, in contrast to no increase in glucose uptake when local forearm arterial glucose concentration was doubled in such a way that the pancreatic arterial glucose concentration remained normal. We do not routinely measure arterial glucose concentration during glucose tolerance tests. A search of the literature failed to reveal previous reports. Note that arterial glucose concentrations at the high glucose concentrations in response to oral glucose are substantially higher (1–1.6 mmol/l [20–30 mg/dl]) than venous concentration; tissues are seeing a higher glucose concentration than we are accustomed to think they are seeing when we look only at venous concentration. Furthermore, systemic arterial glucose concentration is the same throughout the body, but venous concentration varies according to glucose extraction by the tissues drained by the venous blood sampled.

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