Whole body glucose metabolism

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Zierler, Kenneth. Whole body glucose metabolism. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E409–E426, 1999.—This review describes major factors that, singly or together, influence the concentration and distribution of D-glucose in mammals, particularly in humans, with emphasis on rest, physical activity, and alimentation. It identifies areas of uncertainty: distribution and concentrations of glucose in interstitial fluid, kinetics and mechanism of transcapillary glucose transport, kinetics and mechanism of glucose transport via its transporters into cells, detailed mechanisms by which hormones, exercise, and hypoxia affect glucose movement across cell membranes, whether translocation of glucose transporters to the cell membrane accounts completely, or even mainly, for insulin-stimulated glucose uptake, whether exercise stimulates release of a circulating insulinomimetic factor, and the relation between muscle glucose uptake and muscle blood flow. The review points out that there is no compartment of glucose in the body at which all glucose is at the same concentration, and that models of glucose metabolism, including effects of insulin on glucose metabolism based on assumptions of concentration homogeneity, cannot be entirely correct. A fresh approach to modeling is needed.

distribution; uptake; output; blood flow; alimentation; exercise; anoxia; hormonal control; fatty acids

SCOPE OF THE REVIEW

D-glucose is the transport form of carbohydrate, an important fuel for stoking metabolic engines in all mammalian cells. It is the major substrate for neuronal O2 consumption (104). However, it is not the major fuel for either the beating heart (17) or skeletal muscle, at rest (3) or with mild to moderate exercise (54, 136); free fatty acids (FFA) are. Respiratory quotient (RQ = expired CO2/inspired O2) of healthy young adult men and women at rest in the basal state is ~0.76, implying that carbohydrate supplies less than about one-third and fat more than about two-thirds of the nonprotein substrate metabolized by the whole body (27).

The purpose of this review is to bring together information about major factors that influence the concentration and distribution of D-glucose. It is limited to mammalian glucose metabolism, with emphasis on humans, and to the influence of three states: rest, physical activity (including effects of hypoxia), and alimentation (including effects of fat and plasma FFA concentration). The review does not consider intermediary metabolism except insofar as it is necessary for transfer of glucose from interstitial fluid into cells and for endogenous glucose production. Unless the context requires specification of the optical isomer, “glucose” will refer only to D-glucose, and the descriptive “mammalian” will be assumed for all references to bodily processes, unless stated otherwise.

METHODS FOR STUDYING GLUCOSE METABOLISM IN VIVO

Two methods are gold standards. Although they supply only limited information, they have the advantage of being reliable when carried out with due care. They are easily understood and clearly based on steady-state balance studies. 1) Estimation of glucose uptake by a single organ or region of the body is accomplished by an input-output method, first described and used by Chauveau (22), although often called the Fick principle. Input to the organ or tissue is the product of blood (or plasma) flow and arterial concentration of the substance of interest, such as glucose. Output from the organ or tissue is the product of blood flow and venous concentration. Net uptake or output of the substance of interest is, then, the product of blood flow and arteriovenous (a-v) concentration difference. Such methods are valid only in a steady state in which flow and concentrations are constant, although uptake can be measured from integrated a-v differences during transition between two steady states of glucose concentrations, but not during changes in blood flow (133). One of the powerful advantages of this method when applied to studies of metabolism of forearm skeletal muscle and
adipose tissue lies in the possibility of controlling elements in the composition of inflowing blood by close arterial infusion. Forearm blood flow at rest, with circulation to the hand excluded, is of the order of 1% of cardiac output. This makes it possible to introduce hormones or other potent agents by constant infusion into the brachial artery in locally effective concentrations, and yet any of the agent that escapes into forearm venous blood is diluted ~100-fold in the central circulation so that its circulating concentration outside the forearm is impotent to stimulate unwanted responses and counterregulatory mechanisms that would otherwise confound interpretation of the data. Similarly, substrates such as glucose can be introduced by close arterial infusion into the brachial artery so as to produce local forearm hyperglycemia to an extent that might, for example, double normal blood glucose concentration, and yet the general circulating glucose concentration is increased by only 1%, at most, a concentration too small to evoke counterregulatory responses. 2) Total body glucose uptake, under conditions in which there is no endogenous glucose production, is estimated by the Andres glucose clamp method (4). When glucose uptake is constant, total body glucose uptake must equal the amount of glucose, corrected for urinary loss, that must be infused to maintain constant blood glucose concentration, once the entire volume of extracellular glucose distribution has been accommodated. Endogenous glucose production can be shut off by somatostatin, by insulin, or by sufficient hyperglycemia. If there is endogenous glucose production, one seeks to estimate it by the decrease in plasma glucose specific activity when a tracer amount of radiolabeled glucose is added to the infusion. Translation of that decrease in specific activity into a quantitative statement about endogenous glucose production requires further assumptions that may or may not be valid. Methods 1 and 2 have often been used together.

In efforts to find methods for in vivo study of glucose metabolism that are either less demanding or have potential to supply information unattainable by steady-state input-output or glucose clamp methods, a great deal of work has been spent in proposing and using models. These models, or their equivalent representation in the form of equations, have more parameters than can be measured, or than are measured directly. They cannot be solved, therefore, without additional assumptions, as, for example, that total body glucose is distributed in a small number of compartments, in each of which there are no glucose concentration gradients, and that there is a single transfer rate into and a single rate out of each compartment. These are therefore called lumped parameter models and are usually referred to as compartmental analysis. As we shall see, the assumptions are inaccurate. This does not necessarily invalidate use of such a model, but it must be demonstrated for each model and for each experimental condition in which it is used that the inherent inaccuracies are negligible for its specific use, not a trivial task. For details and examples of models, see, for example, major contributions by Bergman (14, 15), Berman (62), Cobelli (6, 14, 15), and a review by Radziuk (97), who uses compartmental analysis as an element in a broader approach. Space limitation prevents detailed assessment of lumped parameter models.

An interesting and influential lumped parameter model, introduced by Bergman and Cobelli and colleagues (14), is the 'minimal model,' designed to interpret data from a frequently sampled glucose tolerance test. The model claims to yield a measure of sensitivity of the whole body to insulin with respect to glucose uptake and a measure of the rate of glucose uptake stimulated by increased circulating glucose and independent of insulin action (a process called "glucose effectiveness"). Although results of the analyses compared favorably with results obtained in glucose clamp experiments under certain conditions, they do not do so under all conditions. Selection of the minimal model was the result of tests for best fit to data of seven candidate models. The model selected assumes two properties known to be contrary to biological fact: first, it assumes that glucose uptake is a linear function of circulating blood glucose concentration and that the slope of that relationship is an index of insulin sensitivity (when, as we shall see, glucose uptake by cells occurs only via saturable cell-surface glucose transporters, so that the relationship cannot be linear, and there is therefore no single, constant slope); and, second, it claims that extrapolation of the best-fit straight line back to intersect the glucose uptake axis measures insulin-independent glucose uptake (when, of course, there cannot be any glucose uptake if blood glucose concentration is zero). One of the problems with the process by which the decision to accept the model was made is that the seven models tested failed to include a Michaelis-Menten model in which the parameters could be sensitive to insulin, a model long used in studies of insulin action on glucose uptake by isolated cells and tissues. A Michaelis-Menten model is an oversimplification, but at least it avoids the two erroneous assumptions (although not the lumped parameter assumption) of the minimal model pointed out above.

We shall see that there are no anatomic regions in the body in which the concentration of glucose is everywhere the same, not even circulating blood plasma. Therefore, strictly speaking there are no compartments, in the sense in which the word is used to mean some volume in which there are no glucose chemical potential gradients, although there may be conditions that are of a scale at which the real chemical potential gradients can be ignored. My own policy is to seek to avoid compartmental models on the grounds that they are likely to yield misleading results, that they require assumptions that are likely to be untrue, that there is often no independent test of validity of the results, and that they are often not needed; one may be able to obtain a useful and desirable answer by a different approach that makes no unwarranted assumptions. Elements of a possible alternative to compartmental analysis lumped parameter models are suggested by Mari (82), in which the circulatory system plays a
primary role in glucose distribution, and its role is
equated to the density function of transit time of
urea through the system. In part, this approach was
anticipated by experiments in which the paired-tracer
dilution method was applied to the perfused rat hind-
limb (60).

**GLUCOSE DISTRIBUTION AND CONCENTRATIONS**

Glucose Concentrations Throughout the Body

Erythrocytes. Fetal and neonatal erythrocytes of all
mammalian species are highly permeable to glucose; in
them glucose concentrations in red blood cell water are
about the same as those in plasma water. In all
mammals studied except for porpoise, rhesus monkey,
and human, red blood cell glucose content decreases at
about the same time as fetal hemoglobin decreases (1).
The ratio of red cell water glucose to plasma water
glucose varies widely among mammals, from near zero
(in swine) to near unity (in primates and porpoise),
with many species at ~0.5 (47). Therefore, whole blood
glucose concentration understimates plasma water
glucose concentration, which is the glucose concentra-
tion of interest as the driving force for movement into
interstitial water. In adult humans, glucose concentra-
tion in plasma water is nearly the same as in red blood
cell water over a wide range of blood glucose concentra-
tion, until plasma glucose concentration becomes ex-
tremely high when erythrocyte glucose transporters
are saturated (84).

Jaquez (63) points out that glucose enters human red
blood cells at a rate that exceeds its red blood cell
utilization by a thousandfold. Whatever else its func-
tion may be, erythrocyte glucose does serve as buffer to
damp the amplitude of variations in plasma water
glucose concentration. Even in those species in which
the glucose concentration in red blood cell water is subst-
entially less than in plasma, erythrocytes serve to buffer
plasma glucose. In rats, red blood cells take up glucose
when there is hyperglycemia and store most of it as
glycogen. When there is hypoglycemia, and under the
influence of a β-adrenergic agonist, red blood cell
glycogen is dissimilated to glucose and moves into
plasma (52). In this context, erythrocytes have been
dubbed "circulating hepatocytes."

Transcapillary exchange and interstitial fluid. With
the possible exception of blood capillary endothelial
cells, no d-glucose molecule crosses a plasma mem-
brane except via a d-glucose-specific transporter. There
is no l-glucose transporter, so l-glucose is excluded
from cells and cannot cross the blood-brain barrier (53).
However, both d-glucose and l-glucose move back and
forth between blood plasma and interstitial fluid (126).
We don’t know whether the route of glucose across
capillaries is transcellular or paracellular or both. It is
because l-glucose is distributed in interstitial fluid
(except for brain), despite the fact that there is no
known l-glucose transporter, that we suspect strongly
that d-glucose does not require a transporter to move
between blood plasma and interstitial fluid. Although
details of d-glucose movement across capillaries are
unknown, the process is usually treated as diffusion,
driven only by a glucose concentration gradient across
capillaries, but it may be more complicated.

Glucose is not distributed instantly across capillaries
into interstitial fluid. When glucose is injected intrave-
nously so as to produce arterial blood plasma hyperos-
smolality, the initial response is net water movement
from interstitial fluid into plasma; water crosses capil-
laries faster than glucose. Glucose concentration in
relatively large bodies of interstitial water (including
lymph, transudates, and edema fluid) is approximately
the same as in arterial plasma water (55). However,
glucose concentration in interstitial fluid must be less
than that in arterial plasma water for glucose to diffuse
out of blood plasma. The gradient need not be steep.

Glucose concentration in subcutaneous interstitial
fluid has been assessed by several methods: cotton
wick, microdialysis, microfiltration, needle-type glu-
cose sensors, and equilibration with microfiltration.
Reported values of the ratio of interstitial to venous
glucose range from ~0.4 (104) to ~1, a value inconsis-
tent with diffusion from blood into interstitial fluid, and
therefore suspect (94). In a study comparing three
methods, Schmidt et al. (106) found that all gave a ratio
of ~0.45. The mean of 0.45 is thought to reflect gradi-
ents in glucose concentration; the field detected by the
sensor includes a volume ranging from interstitial face
of a capillary, where glucose concentration is likely
close to that of arterial plasma water, to adipocyte or
myocyte, where interstitial glucose concentration need
be only just larger than that of free glucose in the cell
(near zero) to enter the cell via a glucose transporter. If
these observations are not artifacts, it is erroneous to
treat total extracellular fluid as though it were charac-
terized by only a single glucose concentration. How-
ever, others report, with microdialysis of perfused rat
limbs, that interstitial glucose concentration is about
the same as that in blood (43). As one expects, when
insulin was added to the perfusate, interstitial glucose
collection in skeletal muscle fell to a greater extent
than did blood glucose (43). Obviously, when muscle
uptake is increased, a fall in interstitial glucose
collection must precede that in blood. There is also a
report of interstitial glucose concentrations, by micro-
dialysis in normal young adults, measured separately
in muscle and in fat tissue interstitium under euglyce-
mic and hypoglycemic hyperinsulinemic glucose clamp.
The two interstitial fluid glucose concentrations were
the same throughout. With euglycemia, the ratio of
interstitial to plasma glucose was ~0.7 but dropped to
~0.34 during hypoglycemia; both the absolute and
relative decreases in glucose concentration in intersti-
tial fluid were greater than in plasma, emphasizing
that extracellular fluid cannot be treated as having no
gradients in glucose concentration (79).

Heterogeneity of venous and interstitial glucose con-
centrations. It is common to speak of a “rapidly equili-
brated” d-glucose space, which consists of extracellular
fluid plus red blood cell water, ~20% of body weight, as
though at any instant there is only one glucose concentration throughout it. However, glucose is not distributed instantaneously throughout its rapidly equilibrated volume. Time is required for glucose molecules to reach systemic capillaries when introduced either into hepatic or renal venous circulation by endogenous glucose production or into portal venous circulation by intestinal absorption or when administered intravenously. Because tissues remove glucose from arterial blood, venous glucose concentrations are less than arterial, except that hepatic venous glucose concentration may exceed arterial during periods in which the liver is adding glucose to the circulation. Venous glucose concentrations are not all the same; they depend on arterial flow rate and the rate of glucose uptake by the tissue drained by the vein in question. In normal young men and women, mean arterial blood glucose concentration in the basal state was 5.0 ± 0.05 mM; forearm arterial-deep venous concentration difference was 0.14 ± 0.017 mM, and forearm arterial-superficial venous concentration difference was 0.19 ± 0.027 mM (9). These are basal state glucose extraction fractions of only 3 and 4%, from which we may conclude that for some restricted purposes it may be acceptable to treat blood plasma glucose concentration as being the same everywhere in a well-defined basal state. However, in response to a maximum dose of insulin, forearm arterial-deep venous glucose concentration difference increased to 1.36 mM, an extraction fraction of 27%, 9 times greater than in the basal state (2). It is, therefore, not accurate to speak of blood glucose concentration as though at any instant under any circumstance it is everywhere in blood the same under all conditions.

Intracellular glucose. It has not been easy to determine whether there is free glucose, as opposed to its phosphorylated products, in most cells, particularly in skeletal muscle. The difficulty is that one seeks to measure a small difference, if any, between total glucose in the sample and extracellular glucose. Most evidence points to the likelihood that, over the range of normal blood glucose concentration, if there is any free glucose in skeletal muscle cells it is below the resolving power of our methods. For example, in rats under glucose clamp at various glycemic levels, no free glucose was detected in skeletal muscle but was present in liver, its concentration varying directly with serum glucose (130). Katz et al. (65) concluded that there is no free intracellular glucose in biopsied human quadriceps femoris muscle, but their conclusion is uncertain because it was based on an assumption that extracellular glucose concentration is the same as that in plasma (so that intracellular glucose tended to be underestimated, although perhaps only negligibly) and on an arguable assumption that extracellular water is 300 ml/kg dry weight of muscle, perhaps an underestimate (so that intracellular glucose tended to be overestimated). That there is very little or no free intracellular glucose in skeletal muscle at normal plasma glucose concentrations implies that glucose is phosphorylated in muscle about as rapidly as it is translocated into the cell. Daily Range of Blood Glucose Concentration

At any moment, the change in concentration of glucose in any small volume element of blood plasma depends on the rate at which glucose is introduced into circulating blood, the rate at which it is distributed throughout the blood circulation, the rate at which it is apportioned between red blood cells and plasma, and the rate at which it is removed from circulation. The limiting event on rate of glucose removal from the circulation is its rate of entry into cells. The rate of glucose entry into cells may be limited by transmembrane transport or by glucose phosphorylation. All of these factors are changing throughout the day in relation to meals, physical activity, emotional state, and stage of development.

Arterial blood plasma glucose concentration is not tightly controlled. In normal young adults, during the course of a day’s activity, arterial blood glucose concentration may vary from a low of a little more than 3 to a high of 9 mM (111). The highs and lows of blood glucose concentration are affected by dietary carbohydrate and fat, by blood plasma levels of FFA, by muscle exercise, by local hypoxia, and by families of hormones.

GLUCOSE TRANSPORTERS: CATEGORIES AND DISTRIBUTION

There are two broad categories of glucose transporters: Na+ dependent (SGLT) and Na+ independent (GLUT). There are several reviews of SGLT (124) and GLUT (87, 91) to which readers are referred, and which serve as references in this section for material not specifically referenced.

Na+ Dependent (SGLT)

By a mechanism not understood, SGLT glucose transport is coupled to downhill Na+ transport, which is thought to confer sufficient energy on the system to permit glucose to cross the cell membrane against its concentration gradient. When loss of glucose from isolated intestinal epithelial cells is prevented by blocking GLUTs in the basolateral membrane, the cell can concentrate glucose, taken up via its SGLT, by ~30-fold (70). SGLTs are limited to some epithelial cells; our concern in this review is mainly with SGLT in the brush border of intestinal and proximal renal tubular cells and in cytoplasmic vesicles close to or attached to the brush-border plasma membrane, at least of small intestine enterocytes.

Na+ Independent (GLUT)

In contrast to the limited distribution of SGLT, GLUTs are distributed widely among nearly all cells, although with varying density. Net movement of glucose across a cell membrane via GLUT is in the direction of a glucose concentration gradient. Because movement down a chemical potential gradient suggests diffusion, but the rate at which glucose travels from one side of the membrane to the other is faster than can be
explained by diffusion of a molecule of its size and hydrophilicity across unit area of a plasma membrane, the process is called facilitated diffusion. This may be a misnomer. GLUTs are not simply pipes through the plasma membrane through which random molecular movement of glucose in aqueous solution drives glucose concentrations in two bulk aqueous phases toward equality, a process that defines “diffusion.” As is thought the case for ion channels, there may be energy barriers in GLUTs that glucose molecules must overcome in connection with one or more association-dissociation steps.

There are six functional GLUT isoforms, of which five transport primarily D-glucose: GLUT-1, GLUT-2, GLUT-3, GLUT-4, and GLUT-7. All GLUTs have considerable homology but vary in their affinity for glucose. To reach cytoplasm of neurons in the brain, glucose has to cross two barriers: the choroid plexus blood-brain barrier via GLUT-1 and the neuronal plasma membrane via GLUT-3. GLUT-1 is the most widely distributed, serving many cell types alone or in company with another isoform. It is in pancreatic β-cells in humans, whereas GLUT-2 serves that role in rodents (34), illustrating the fact that we cannot necessarily extrapolate from one species to another. GLUT-2 is also the major GLUT for intestinal absorption and renal reabsorption and for releasing glucose from and translocating it into hepatocytes. GLUT-4 is the major GLUT in those cells that respond to insulin by an increase in glucose uptake. In adipocytes and muscle cells, most GLUT-4 is located in the wall of intracellular vesicles in the basal state, with only a little in the cell surface plasma membrane. In skeletal muscle there may be nearly none in the plasma membrane in the basal state. GLUT-7 has been localized to hepatocyte endoplasmic reticulum; it is conjectured that GLUT-7 may serve to transport newly produced glucose out of endoplasmic reticulum lumen into cytoplasm, from which it leaves the cell for the bloodstream by way of GLUT-2 (119).

GLUT isoforms vary in their transport efficiency. Numerical values for the Michaelis constant, K_m, have been estimated for the four GLUT isoforms found in cell surface membranes. They fall into three groups. It is not known to what extent GLUT K_m in purified or semipurified systems accurately reflects the affinity between the GLUT isomorph and glucose in situ. Nevertheless, we may get an idea about relative values: the lowest K_m is 1–5 mM, for GLUT-3, the brain GLUT, consistent with the observation that increased glucose concentration in blood perfusing the brain does not increase brain glucose uptake; the highest K_m is 20–40 mM, for GLUT-2, which may have to deal with intracellular glucose concentrations in enterocytes, renal tubular cells, and hepatocytes that are higher than plasma levels; GLUT-1 and GLUT-4 K_m falls within the middle-to-upper range of normal blood glucose concentration. These numerical K_m values should be taken with reservation; it is not clear that the kinetics of glucose transport via GLUT obey the Michaelis-Menten assumptions in detail.

SGLT and GLUT Cooperation and Mobilization

SGLT and GLUT-2 work in series in small intestinal enterocytes and in the renal proximal tubule. In both cases, SGLT is on the brush-border luminal membrane, where it absorbs glucose from gut contents or from renal glomerular filtrate; absorbed glucose travels somehow through cytoplasm to the basolateral membrane, through which it travels via GLUT-2 to interstitial fluid and the portal vein in the gut and to the renal vein in the kidney.

Enterocyte SGLT occurs not only in the brush-border membrane but also in, or in the membrane of, intracellular vesicles. These vesicles fuse with the brush-border membrane to increase surface SGLT, which subsequently cycles back to the cell interior (129). Similarly, in adipocytes and cardiac and skeletal muscle, GLUTs are found laced in the wall of intracellular vesicles. Under circumstances to be described later, these vesicles are mobilized to fuse with the cell surface membrane, increasing plasma membrane GLUT density (29, 114). Subsequently, cell surface GLUT returns to the cell interior via endocytosis. In adipocytes, the intracellular GLUTs are both GLUT-1 and GLUT-4, and both are recruited to the cell membrane (137). In skeletal muscle, the predominant sarcolemma GLUT in the basal state is GLUT-1. The smaller amount of GLUT-4 in the basal state is overwhelmingly (38) or exclusively in the wall of the transverse tubule (T tubule) (42). The intracellular GLUT is exclusively GLUT-4, or nearly so, and, in response to stimuli that mobilize the intracellular vesicles to fuse with cell membrane, they appear to dock only, or at least mainly, with T tubules (42, 81). There are quantitative differences in these responses among muscle fibers (48). Except for adipocytes and cardiac and skeletal muscle, GLUTs have been identified only in the cell surface plasma membranes.

RELATION OF GLUCOSE METABOLISM TO ALIMENTATION

The Food and Famine Hypothesis

The food and famine hypothesis considers effects of meals and periods between meals on blood glucose concentration and glucose uptake and output as determined by various combinations of hormones throughout the day (96). The hypothesis treats three periods: prandial and immediate postprandial, delayed postprandial, and remote postprandial, which evolves into the basal state or postabsorptive period. These periods are characterized by nearly reciprocal relations between blood plasma concentrations of, on the one hand, insulin, the major stimulator of glucose uptake, and, on the other hand, growth hormone (GH), β-adrenergic agonists, and glucagon. GH opposes insulin-stimulated glucose uptake. Glucagon and β-adrenergic agonists stimulate glucose production via glycogenolysis and gluconeogenesis.

Exposure to insulin, which dominates in the prandial and immediate postprandial period, encourages storage of carbohydrate and fat and reduces or prevents
hepatic glucose production. Exposure to GH plus insulin in the delayed postprandial period encourages protein synthesis. At the same time, because the antilipolytic effect of insulin prevails over the lipolytic effect of GH, antilipolysis continues. In a series of reports, Froesch’s laboratory has added a role for insulin-like growth factor I (IGF-I) (summarized in Ref. 85). Exposure to GH and IGF-I, with basal concentrations of insulin, in the remote postprandial period encourages mobilization and peripheral oxidation of fat and retards translocation of glucose into muscle and adipose tissue. IGF-I has an “insulin-sparing” effect. The combination of insulin and IGF-I infusions during a euglycemic clamp caused greater whole body glucose uptake than insulin alone. Whereas GH reduces the glucose uptake response to insulin, IGF-I increases it. IGF-I diminishes the GH inhibition of insulin effect on glucose uptake. On the other hand, IGF-I, like GH, is lipolytic, and IGF-I enhances GH in its prevention of protein catabolism. Exposure to increasing concentrations of β-adrenergic agonists and glucagon in the intermediate and late postprandial period causes glycogenolysis and gluconeogenesis.

Dallman et al. (30) considered interactions between insulin and corticosteroids, via the hypothalamic-pituitary-adrenal axis, in response to fed and fasting cycles. Corticosteroids may be either synergistic with or antagonistic to insulin-stimulated glucose uptake, in a concentration-dependent manner. Dallman’s review should be consulted for analysis and summary of the complex interplay.

The Basal State and Remote Postprandial Period

Distribution of glucose uptake and output. The basal state is simply a quite late postprandial period, or early starvation, in which quasi-stability is maintained for some properties. In the basal state in humans, insulin and GH blood levels are at or near their lowest concentration of the 24-h period, insulin at 5–10 U/ml (30–60 pm) and GH at 5 ng/ml (30, 59).

There is a slow decline of blood glucose concentration, by less than 1%/h in the basal state (3, 59), so that total body glucose uptake must exceed total body glucose production in the basal state. We can construct only an incomplete balance sheet for basal glucose uptake and production in humans. Endogenous glucose production (by glycogenolysis and gluconeogenesis; all data in mmol·min⁻¹·1.73 m² body surface⁻¹) by the splanchnic bed would be 0.62 (89), by the kidney 0.07, and total production 0.69 (113). Glucose uptake by brain would be 0.43 (104), by skeletal muscle 0.12 (3), by heart 0.08 (116), and the total by these three tissues 0.63, or ~90% of reported glucose production. Not available are data for other tissues, particularly skin and adipose tissue, large masses of tissue, which, along with cumulative uncertainties about the calculations reported, must make total uptake slightly exceed total basal production.

Fate of glucose. If all glucose taken up were oxidized completely to CO₂ and H₂O (which it is not), the total glucose uptake could account for only 35% of the observed O₂ uptake. This predicts a total body RQ of 0.8. The actual RQ is lower, ~0.76, because glucose is not oxidized completely.

Brain glucose uptake, if oxidized completely, can account for more than (~125%) of the observed O₂ uptake (101), which suggests that some glucose is not oxidized completely in the brain. Although adult brain depends entirely on glucose oxidation in the basal state, that is not true for the embryo. Embryonic brain oxidizes ketones, not glucose, because it lacks, or poorly expresses, GLUT-1, which is necessary for glucose to cross the choroid plexus. In the rabbit, brain GLUT-1 is upregulated through days 14–70 (39).

Oxidation of glucose uptake by the beating heart accounts for only 35% of observed O₂ uptake (116). Unlike skeletal muscle, the heart takes up lactate and can use it as substrate for oxidation. This still leaves most of the O₂ uptake to be accounted for by FFA (17).

Between 40 and 60% of observed forearm muscle glucose uptake in the basal state is accounted for by lactate production (3). This implies that, at any instant, some 40% or more of forearm muscle fibers are not perfused, but presumably no fiber is unperfused for long. That is, blood circulation to resting muscle, ~3–4 ml·min⁻¹·100 ml forearm volume⁻¹, is small and limited, so that it is economically distributed among forearm muscle fibers, now perfusing one group of fibers and later another group. (Lactate, derived from skeletal muscle anaerobic glycolysis, released into the circulation, is used as a substrate for myocardial O₂ consumption and for hepatic glycogen synthesis.) When glucose uptake is corrected for lactate production, the remaining forearm muscle glucose uptake accounts for only ~16% of forearm muscle O₂ uptake. This value predicts a forearm muscle RQ of 0.74. The observed RQ was 0.76 ± 0.02. The remaining 84% of forearm O₂ uptake in the basal state is accounted for by FFA extracted from blood (8). This calculation means that little or none of the glucose taken up by muscle in the basal state is stored as glycogen (3), a fact demonstrated by nuclear magnetic resonance spectroscopy (107).

Difference between in vivo and in vitro muscle glucose uptake. Glucose uptake by the forearm in the basal state is only ~0.5 μmol·min⁻¹·100 ml of forearm⁻¹, or an estimated 390 nmol·h⁻¹·g forearm muscle⁻¹ (2). Excised rat hemidiaphragm muscle takes up glucose at a rate ~50 times greater than that (134), and excised rat epitrochlearis muscle takes up glucose at ~20 times the rate of the human forearm (21). Skeletal muscle responds to hypoxia and to contraction by rapid increase in the number of GLUTs on its surface. Because skeletal muscle contracts the instant its nerve is cut and because there is apt to be a period of hypoxia during dissection, it is possible, even likely, that estimates of GLUT density in excised muscle in the “basal” state are actually higher than true basal, owing to the insults of dissection with consequent translocation of GLUT to the surface membrane. We shall see, later, that the stimuli of contraction and, possibly, hypoxia during preparation of muscle for in vitro experiments.
are probably submaximal, because in vitro muscle dose increase glucose uptake in response to either electrical stimulation or hypoxia (7, 35). When this excised muscle is then exposed to insulin at concentrations that elicit a maximum response, total cell surface membrane GLUT may or may not increase by as much absolute amount as it does in normally perfused muscle, but the ratio of glucose uptake with to that without insulin will be smaller than in situ perfused preparations owing to the abnormally high basal value. Kern et al. (67) demonstrated this phenomenon by comparison of glucose uptake in excised strips of rat skeletal muscles with that by the same muscles subjected only to anesthesia and hindlimb perfusion. Basal transport was lower and maximum transport in response to insulin was higher in perfused muscles, so that the glucose uptake ratio in response to insulin was much higher in perfused than in excised muscles (15 vs. 2.5 in soleus; 9.8 vs. 2.3 in extensor digitorum longus). Some investigators are now preincubating excised skeletal muscle for 30 min or longer, during which time it is hypothesized that the abnormally high density of plasma membrane GLUT (in response to the stimuli of anesthesia and excision) is reduced to normal, perhaps by recycling of surface GLUT. Glucose transport measurements after preincubation are then lower and approach those of muscle in situ.

Prandial State

Glucose absorption. Readers are referred to reviews for further details (41, 59, 64, 93). Glucose in the gut is absorbed mainly in the jejunum and ileum, by two routes: transcellular and paracellular. The paracellular route is across the tight junction between enterocytes, into lateral clefts between cells. The transcellular route was described above, via SGLT and GLUT-2. An increase in dietary carbohydrate increases glucose absorption from small intestinal segments via an unknown path that leads to an increase in the number of SGLT in the brush-border membrane (40, 64). It is inferred that SGLT-1, by successive exo- and endocytosis, cycles between the interior and the plasma membrane. Little is known yet about this cycling process (41). High concentration of glucose in the gut lumen also increases paracellular glucose transport by an unknown mechanism, possibly by a cascade of signals from brush-border SGLT, which may be the only available glucose sensor (93).

Translocation through GLUT-2 in the basolateral membrane is increased by hyperglycemia within 30 min by an unknown mechanism (24). Increase in glucose transport exceeds the increase in number of GLUTs, so that it was suggested that the intrinsic transport activity of GLUT was increased as well as the GLUT number. There is also a slow increase in basolateral GLUT number after ~3 days in response to increased dietary carbohydrate (23), probably due to increased message and synthesis, combined with slow migration of enterocyte from the crypt. Paths for stimulating this GLUT response are unknown.

Effect of prandial hyperglycemia: insulin-dependent and -independent glucose uptake. Glucose absorption generates the familiar prandial blood glucose curve. The rapidity of rise in blood glucose concentration and the height of its maximum concentration depend on the rate at which the stomach empties its contents, because most glucose absorption occurs in the jejunum and ileum. The rate of gastric emptying is well controlled. Teleologically, it makes sense that hypoglycemia actually accelerates gastric emptying and hyperglycemia retards it. Two peptides decrease the rate of gastric emptying: glucagon-like polypeptide-1 (GLP-1) (123, and for review 37) and amylin (127). It is suggested that the effect of GLP-1 on gastric emptying is mediated via vagal afferents (61). Amylin is a peptide hormone, cosecreted with insulin from pancreatic β-cells. Its mode of action is not known.

Hyperglycemia declines as glucose is transferred from interstitial fluid into cells. Baron et al. (11) and, later, Bergman [Best et al. (16)] emphasized that there are two components to this increased glucose uptake, an insulin-dependent and an insulin-independent component. It is not simple to establish the relative contribution of the two components, and there is some disagreement about the quantitative estimates. Bergman calls the process of insulin-independent glucose uptake glucose effectiveness, but a more fitting designation is “GLUT effectiveness,” for the following reason. Increased glucose uptake has to occur with hyperglycemia, without intervention of hormones, simply because the glucose concentration gradient from interstitial fluid to cytoplasm is greater, and increased facilitated diffusion must occur via all GLUTs that are not saturated, unless hexokinase is limiting. The two tissues that do not increase glucose uptake in response only to increased glucose delivery are brain and, in humans, skeletal muscle (5). Brain does not respond to hyperglycemia because its GLUT-3 has so small a K_m value that it is saturated at euglycemic levels. In situ, muscles of the forearm in humans do not increase glucose uptake when the local (but not systemic) forearm arterial blood glucose level is raised to a steady value of 10–15 mM, presumably because GLUT density in the basal state is so small (5). In methods for studying glucose metabolism in vivo, I wrote that a powerful asset of the forearm input-output technique lies in use of constant infusion into the brachial artery to produce effective local forearm concentrations of the injected material, which are then rendered impotent to have systemic effects because the infusate is diluted by ~100-fold when venous effluent from the forearm mixes with blood in the central circulation. I have been able to locate only one report, an abstract, in which this technique was used to inquire into the effect of a purely local limb hyperglycemic input (5). In six healthy young adult men, brachial arterial glucose concentration was increased by ~50, 100, and 200% above normal by continuous infusion into the brachial artery. There was no effect on forearm blood flow (hand excluded from circulation) or on glucose a-v concentration difference in blood draining forearm muscle; that is, there was no...
The first stimuli for insulin release from pancreatic β-cells are a family of peptides, incretins, related in their amino acid sequences, that include glucose-dependent insulinotropic polypeptide, GLP-1, glucagon, vasoactive intestinal peptide, and pituitary adenyl- dependent insulinotropic polypeptide, GLP-1, glucagon, which stimulates endogenous glucose production. The incretin GLP-1 has multiple roles; not only does it stimulate insulin release, but it also inhibits glucagon release, thereby tending to delay hepatic glycogenolysis.

When hyperinsulinemia becomes sufficiently high, the insulin-receptor complex in adipocytes and in cardiac and skeletal muscle initiates a transduction chain that results in translocation of intracellular vesicles to fuse with the plasma membrane. Laced in the walls of these vesicles are GLUTs, GLUT-1 and GLUT-4 in adipocytes, but almost exclusively GLUT-4 in skeletal muscle (for reviews see Refs. 73 and 75). All studies in which insulin-stimulated increases in glucose uptake and insulin-stimulated increase in cell surface GLUT have both been measured find that the relative increase in glucose uptake exceeds that of plasma membrane GLUT. In particular, the increase in plasma membrane GLUT-1 has always been reported to be substantially less than the proportionate increase in glucose uptake. When plasma membrane GLUTs were assessed by first subjecting the cells to subcellular fractionation by density gradient centrifugation, insulin-induced increase in plasma membrane GLUT-4 was always, and usually substantially, less than the proportionate increase in glucose uptake. With availability of an imper- meant bismannose photolabeling method (25), it became unnecessary to subject cells to subcellular fractionation, and reported values of insulin-induced increases in plasma membrane GLUT-4 in a few, but not most, studies were reported as ~80% of the degree of increase in glucose uptake by rat adipocytes (57, 77) and by 3T3-L1 adipose-like cells (20). There are no reports that the increase in plasma membrane GLUT-4 is proportionately greater than that of glucose uptake.

It is shown by a model-free analysis of a system in which d-glucose cannot enter a cell except via a GLUT and in which it must enter the cell only by either GLUT-1 or GLUT-4, that if the increase in plasma membrane GLUT-1 induced by insulin is proportionately less than that of glucose uptake, which is in fact the case in all reports, then the increase in plasma membrane GLUT-4 must be greater than that of glucose uptake, unless insulin also increases the intrinsic activity of one or both GLUT isoforms to transport glucose (131). Analysis of available reports in which plasma membrane densities of both GLUT isoforms and glucose uptake were measured led to an estimate that the relative increase in GLUT-4 would have to have been about 4 times greater than actually observed if insulin increased glucose uptake only by translocat- ing glucose to the cell surface, and that insulin-stimulated increase in plasma membrane GLUT intrinsic activity accounted for at least one-half of the observed increase in glucose uptake (131). This suggests the possibility that GLUTs, at least GLUT-4 and possibly GLUT-1, in the plasma membrane exist in at least two stable configurations, and that, similar to gated ion channels, they can undergo state of state from one
configuration to another, in which their ability to transport glucose is increased in response to insulin and perhaps to other stimuli.

In humans under glucose clamp experiments, 75% of estimated whole body insulin-mediated glucose uptake during euglycemia is into skeletal muscle, and 95% is during hyperglycemia (11).

The question of whether insulin, under physiological conditions, increases blood flow to skeletal muscle, and whether this increased flow is an additional cause of increased glucose uptake, is controversial. Blood flow of resting skeletal muscle is relatively small and distributed heterogeneously so that some regions are metabolizing anaerobically, producing lactate to account for about one-half the muscle glucose uptake (3). It is conjectured that although about one-half the muscle fibers are not perfused at rest, survival of fibers requires that no fiber is underperfused for long, at most only a few minutes. We envision skeletal muscle circulation as continuously redistributed among resting fibers, so that there are scanning waves of alternating vasoconstriction and dilatation (3). We suppose that this parsimonious blood flow to resting muscle is a device for minimizing resting cardiac output and the work of the heart. There is similar, but less extreme, distribution of blood flow to the myocardium. Unlike the case for skeletal muscle, heterogeneity of coronary flow to the normal beating heart can be relatively stable, for as long as a day. Deussen (33) studied heterogeneity of blood flow and of glucose uptake in the dog heart and concluded that variations in local blood flow cannot explain the differences in local glucose uptake.

Insulin stimulates nitric oxide (NO) synthase in vascular endothelium (105, 112) and therefore is capable of vasodilating. Baron and his colleagues (for review of publications from their laboratory see Refs. 11 and 12) have a series of reports that insulin, administered by continuous intravenous infusion for hours, increases leg blood flow. Laakso et al. (77) obtained insulin-leg blood flow dose-response curves and reported that, in normal young men, the maximum effect, an $\sim 70\%$ increase in leg blood flow, occurred at a systemic circulating serum insulin concentration of $\sim 600$ pM (100 mU/ml). Half-maximum response occurred at $\sim 300$ pM (50 mU/ml).

It has been questioned whether this mechanism for increasing blood flow to an arm or a leg is physiological, for the following reasons: 1) The effect of insulin on blood flow is remarkably slow. In a euglycemic hyperinsulinemic clamp study in which serum insulin concentration was held at $\sim 530$ pM, leg blood flow had not quite reached its full response after 4 h (77). Insulin-induced increase in limb blood flow has been confirmed when insulin is administered intravenously, usually in hyperinsulinemic glucose clamp studies, but only after hours 4–6 (125). This delay suggests two grounds for concern about the physiological significance of the observation. First, at no time during the course of a day is there normal hyperinsulinemia for a period as long as 4 h; prandial hyperinsulinemia is transient; it reaches a peak in about half an hour or so after a glucose meal, the peak is sustained for perhaps 15 min, and the total duration of prandial hyperinsulinemia is $\lesssim 2$ h, most of it below insulin levels found to affect blood flow when administered by close arterial injection. Interpolation of the data of Laakso et al. (77) suggests that maximum transient prandial hyperinsulinemia would be expected to increase limb blood flow, if at all, by $\lesssim 10\%$ in the time available for an effect, a value unlikely to be demonstrable by present methods. Second, if the mechanism by which insulin increases blood flow to an arm or a leg is stimulation of NO synthase in endothelium of limb vasculature, why is there such a delay? Other vasoactive agents administered intra-arterially act almost immediately and can produce much greater increase in local blood flow, for example, infusion of adenosine into a brachial artery (89) and infusion of sodium nitroprusside (90). Although it has been demonstrated that administration of N-monomethyl-$\omega$-arginine, a competitive inhibitor of NO synthase, prevents increased blood flow otherwise seen with intravenous insulin after several hours, the delay of limb vasodilator response to sustained intravenous insulin suggests that insulin sets in motion some systemic response that leads to increased limb blood flow under these artificial conditions; that is, the increased limb blood flow in the presence of several hours of intravenous insulin infusion is unlikely to be attributable to a direct action of insulin on resistance vessels in the limb. 2) The second kind of observation that questions the physiological verisimilitude of reported limb vasodilatation in response to several hours of intravenous insulin is that, when insulin is administered by intravenous infusion, there is either no vasodilatation at a small dose of insulin or a relatively small increase, $\sim 20\%$, in blood flow after several hours of a large dose (see Refs. 115 and 125 for additional references). Reexamination of our data on insulin effect on brachial arterial blood flow fails to show an effect of insulin, although a $10\%$, and in some instances even a $20\%$, change in blood flow could not be detected (2, 135). We infused insulin at constant rate into a brachial artery for only 26 min, less time than has been necessary to demonstrate insulin-induced increased blood flow, but with that infusion, forearm glucose uptake increased by 14-fold. When increased limb blood flow has been observed to accompany longer intra-arterial infusions of insulin, unfortunately in none of the experiments has the recirculating insulin concentration been measured. Because only 10–20% of intra-arterial insulin is extracted by forearm tissues, recirculating insulin concentration could become significantly high after several hours, so that it is possible that, when intra-arterial insulin infusion does lead to increased flow, the vasodilator may have been produced in response to systemic, rather than local, hyperinsulinemia. Again, if limb muscle vasodilatation in response to insulin is caused by NO synthesized by muscle vascular endothelium, why is there no, or only slight, vasodilatation in response to intra-arterial infusion?
This is a review of glucose metabolism, not of insulin actions. Our concern here is only to address the question of whether an increase in blood flow can be responsible for increased local glucose uptake. Yki-Järvinen and Utriainen (125) point out that there is increased forearm glucose uptake in response to intra-arterial insulin infusion within minutes, whereas, they say, a vasodilating response to intravenous insulin requires some 4–6 h. Furthermore, the responses are disproportionate: more than a 10-fold increase in glucose uptake for, at most, a doubling of blood flow. Limb blood flow can be increased by other means, for example by intra-arterial infusion of adenosine (89) or of sodium nitroprusside (90), with no effect on glucose uptake. However, Baron (10) proposes that there is nonnutritive blood flow to skeletal muscle, functional arteriovenous shunts, and that insulin vasodilates potentially nutritive arterioles, thereby recruiting capillary beds and increasing capillary surface through which glucose can diffuse into interstitial fluid. Baron’s conjecture has received some experimental support by Vicini et al. (118), who used positron emission tomography (PET) to estimate regional blood flow distribution in the leg before and during euglycemic, hyperinsulinemic clamp. They conclude that, although they could not detect an insulin effect on the relative dispersion of blood flow, the data suggested that insulin vasodilated fewer perfused areas to a greater degree. A similar technique was used by Raitakari et al. (98) and by Utriainen et al. (117). The latter group asked if the degree of glucose uptake in small regions of femoral muscle correlated with the blood flow to the region. Analysis of PET in the basal state showed no colocalization of the flow marker $^{15}$O$\cdot$H$_2$O and the glucose marker $^{18}$F$\cdot$2-deoxy-D-glucose ($^{18}$F$\cdot$2-DG), both given by intravenous infusion. When insulin was given intravenously for 60 min, there was a 30% increase in blood flow, increased heterogeneity (a larger standard deviation about the mean flow), but about the same coefficient of variance as in the basal state, and regions of increased flow colocalized with regions of increased glucose uptake. This colocalization is not surprising. I referred earlier to evidence that GLUTs in forearm muscle do not increase glucose uptake in response to glucose load created by increasing the local forearm blood glucose concentration, and that this implies that skeletal muscle surface GLUTs in the basal state are in such low concentration that they are saturated at normal blood glucose concentration (5). If this is correct, then an increase in glucose delivery caused by increasing local blood flow to muscle should not increase glucose uptake, unless the increased blood flow were to regions not previously perfused. We assume, then, that when forearm intra-arterial infusion of vasodilators, such as adenosine and sodium nitroprusside, increases blood flow but does not increase glucose uptake, there has not been increased flow to previously underperfused regions, and that when intravenous insulin ultimately causes increased muscle blood flow and increased glucose uptake, in addition to insulin’s recruitment of GLUTs to the muscle surface (which long precedes increased blood flow), there may now be improved perfusion of previously underperfused regions in which muscle surface GLUTs had not previously been saturated.

Although an increase in limb blood flow in response to intravenous insulin has been correlated with insulin-stimulated glucose uptake, the time course of the two responses is different. With a hyperinsulinemic clamp, increased glucose uptake is at half-maximum in ~40 min; increased limb blood flow is at half-maximum in ~100 min (77). This sizable difference in time course makes it unlikely that increased blood flow, even under these artificial conditions, had any role in increased glucose uptake. Utriainen et al. (117) studied a group of normal men and a group of men with non-insulin-dependent diabetes mellitus (NIDDM) who were resistant to insulin with respect to glucose uptake. Mean blood flow and the dispersion of blood flow, both in the basal state and in response to hyperinsulinemic clamp, were the same in the two groups, although glucose uptake by the diabetic group in response to insulin was subnormal. It can be concluded that, at least in this group of diabetic patients, the increase in limb blood flow and its distribution in response to systemic insulin were insufficient to cause increased glucose uptake.

In summary, although circumstances can be established in which insulin increases blood flow to skeletal muscle, these circumstances are not physiological. Insulin infused intra-arterially for sufficiently long to increase forearm glucose uptake does not increase forearm blood flow, or produces only a small increase. Furthermore, insulin-stimulated glucose uptake occurs more rapidly than the increase in blood flow. Critical experiments are still needed to settle the question of the role, if any, of insulin-stimulated vasodilatation. If our concept of the distribution of muscle blood flow is correct, waves of asynchronous vasoconstriction, alternating with vasodilatation, scan muscle so that at any instant about one-half the muscle fibers are forced to metabolize anaerobically. What we may need is an instrument not yet invented, a cinematic PET, to inform us about blood flow distribution and glucose uptake distribution as functions of time.

Although there is evidence that insulin stimulates NO synthesis in endothelial cells, and that this NO may vasodilate and increase blood flow, there are reports that NO can have effects on glucose uptake independent of blood flow. Excised rat skeletal muscle was exposed to either a maximum concentration of insulin or to electrical stimulation to contraction. A NO synthase inhibitor decreased basal and exercise-stimulated glucose uptake but had no effect on insulin-stimulated glucose uptake. Addition of the NO donor, Na nitroprusside, increased basal glucose uptake; this effect was additive to the effects of submaximal and maximal insulin concentrations (8). In a related report, two NO donors increased basal glucose uptake of excised rat soleus muscle. This effect was additive to and independent of insulin concentration-dependent increase in glucose uptake (128). These reports support the conjecture that insulin and exercise act on separate...
GLUT pools. It is further suggested that NO might be produced by skeletal muscle during contraction, and perhaps also during hypoxia, which (see below) seems to draw on the same GLUT pool as contraction.

When insulin concentration is high and glucagon and epinephrine concentrations are low, hepatic and renal glycogenolysis and gluconeogenesis cease. Whereas in the basal state little or none of skeletal muscle glucose uptake can be accounted for by glycogen deposition, in response to insulin ≥70% of glucose uptake is accounted for by an increase in muscle glycogen (2, 107).

Insulin stimulates much greater multiples of increase in skeletal muscle glucose uptake in the intact subject than in excised muscle or its membrane fractions. As stated earlier in Difference between in vivo and in vitro muscle glucose uptake, basal glucose uptake in excised muscle is much greater, probably artifically, than in normally perfused muscle in situ. It is not surprising, then, to find that in excised skeletal muscle preparations (120, 132) or plasma membrane vesicles from skeletal muscle (48), insulin increases glucose uptake by only about 2- to 3-fold compared with a 5-fold (74) to 15-fold (67) increase in glucose uptake in perfused hindlimb muscles of the rat, anesthetized and exposed to a surgical procedure, and compared with an ~15-fold increase by forearm muscles of a human in response to constant intrabrachial artery insulin infusion (135). These observations emphasize the fallacy of relying on multiples of increase to compare effectiveness of various agents.

Role of fatty acids in modifying glucose metabolism.

ON GLUCOSE UPTAKE. On the basis of their observation that FFA decreased carbohydrate (CHO) oxidation in rat diaphragm and perfused heart, Randle et al. (99) proposed, in 1963, that circulating FFA led to increased FFA uptake, increased FFA metabolism with increased production of acetyl-CoA and citrate, followed by the known acetyl-CoA inhibition of pyruvate dehydrogenase, which decreases glucose oxidation, and the known citrate inhibition of phosphofructokinase, thereby blocking glycolysis (99). This process was considered to proceed independently of any hormonal control. It has been confirmed repeatedly that an increase in plasma FFA increases fat oxidation and decreases CHO oxidation and that this occurs in proportion to the plasma concentration of FFA over its physiological concentration range (18). However, there is disagreement as to whether the Randle cycle is the underlying mechanism. It is generally agreed that, in the presence of high plasma FFA, insulin-stimulated glucose uptake is reduced. Resistance to insulin in patients with NIDDM has been associated with high plasma FFA concentration (51). Mechanisms underlying this insulin resistance are unknown. In rats on a high-fat diet, plasma insulin concentration was higher than in rats fed a high-CHO diet, presumably a reactive hyperinsulinemia in response to peripheral resistance to insulin. GLUT-2 and glucokinase mRNA levels in pancreas from high fat-fed rats were only one-half as great as in high CHO-fed rats, suggesting the possibility that hyperglycemic signaling to insulin release from β-cells may be impaired in high fat-fed rats (68). Skeletal muscle from rats on a high-fat diet translocated as much GLUT-4 to the plasma membrane but transported less glucose than rats on a high-CHO diet, suggesting that the effect was on the hypothetical activation of surface membrane GLUT rather than on its transport (103). Most recently, from hyperinsulinemic-euglycemic studies in conscious rats infused with fatty acids, data appear to confirm that the Randle glucose-fatty acid cycle is the predominant underlying mechanism for FFA interference with insulin-stimulated glucose uptake (69). In a related study, fatty acids were infused into rats to maintain 24-h elevation of plasma FFA. Under euglycemic-hyperinsulinemic clamp, there was less glucose uptake in the high-FFA group in response to insulin by an unidentified mechanism (80).

Perhaps the most compelling support of Randle's hypothesis (66) in humans comes from input-output studies of legs in healthy young adults, coupled with analyses of biopsied muscle and euglycemic insulin clamps. When FFA serum was kept constant during hyperinsulinemia by FFA replacement, compared with findings when insulin was permitted to reduce FFA levels, there was less glucose uptake, less glycogen stored (i.e., reduced glycogen synthase activity), reduced leg respiratory quotient, and reduced muscle pyruvate dehydrogenase activity, all in line with Randle's hypothesis.

ON ENDOGENOUS GLUCOSE PRODUCTION. Insulin decreases or prevents net release of glucose from the liver, and perhaps the kidney, by two mechanisms, direct and indirect.

Direct effects of insulin on liver and kidney are exerted by way of insulin's actions on the insulin receptor, initiating transduction cascades that lead to increased glycogen synthase activity and decreased glycogenolysis (110).

It was first observed in 1987, during studies of obese subjects, that increased systemic insulin concentration, in the estimated absence of increased portal venous insulin concentration, suppressed hepatic glucose output (95). The phenomenon was confirmed and demonstrated convincingly in normal animals, including humans, by a series of reports mainly from the laboratories of Giacca (McCall et al., 83), Bergman (Mittleman et al., 86), and Cherrington (Sindelar et al., 108). A contributory factor is insulin inhibition of glucagon release from the pancreas.

There is general agreement that the major factor in insulin's indirect suppression of endogenous glucose production is decreased plasma FFA concentration, due to the antilipolytic effect of insulin on adipose tissue. When fatty acid is infused during insulin administration so as to maintain normal circulating FFA concentration, hepatic glucose output is at least partly restored (78, 101, 109). The mechanism by which reduced FFA concentration suppresses hepatic glucose output is unknown. Sindelar et al. (109) report that increased peripheral insulin concentration causes a small decrease in hepatic uptake of gluconeogenic substrate,
prevented by fatty acid infusion. This group of investigators found increased intrahepatic lactate concentration accompanying insulin-induced anti-lipolysis, and they propose that decreased circulating FFA somehow suppress glucose-6-phosphatase activity so that hepatic glycogenolysis results in increased lactate production instead of the usual glucose production.

There is disagreement about the relative importance of the contribution of the indirect effect of insulin in suppressing hepatic glucose output. Bergman's group (86) calculates that most of insulin-induced suppression is indirect, whereas Cherrington's group (108) calculates that most of the effect is directly on the liver via the hepatic insulin receptor. The two groups use different experimental designs. Cherrington's design, including input-output measurements across the portal circulation, glucose clamp, and pancreatic clamp (suppression of insulin and glucagon secretion), provides more complete control of variables.

**EXERCISE**

In his century-old input-output studies of metabolism of the levator of the upper lip of the horse, Chauveau caused exercise of that muscle by feeding the horse oats (22). He found increased blood flow, increased glucose uptake, and, later, increased lipid uptake. Although there was a lapse of about 70 years before Chauveau's work was confirmed and extended, it was well known in the 1940s that severe muscular work caused a drop in blood glucose concentration (92).

Readers are referred to reviews for further details (49, 58, 122). During brief periods (e.g., ~20 min) of mild-to-moderate exercise, glucose uptake increases but blood glucose concentration tends to remain constant because endogenous glucose production matches the increased uptake (54, 136). During this period, insulin release is inhibited, and glucagon and catecholamine releases are increased, accounting for the increased hepatic glucose production. Despite increased muscle glucose uptake, the RQ of exercising muscle, both leg (54) and forearm (136) in humans, remains about the same as at rest, between 0.68 and 0.79, as does total body RQ, because FFA uptake is also increased proportionately (136). Despite increased muscle uptake, plasma FFA levels rise during exercise (54), perhaps due to decreased plasma insulin with loss of its antilipolytic effect and to increased circulating lipolytic agents (71).

It is difficult to study metabolism quantitatively during strenuous exercise, because a steady state cannot be maintained for long. RQ at the lungs is an invalid measure of the distribution of oxidized substrate, because the lactic acidosis of exercise increases blood CO₂. Nevertheless, it is reported that, during strenuous exercise, not only is glucose the major substrate for oxygen consumption, but also that strenuous exercise is not possible without adequate glycogen stores (58). Although authors write of metabolic effects of exercise or of muscle contractions, it should be noted that it has not been possible to measure metabolic responses during the period of exercise or of muscle contraction in many experiments. For example, when muscles are stimulated to contract in situ and then excised for metabolic studies in vitro, or excised muscles are stimulated to contract and then submitted for metabolic analysis, the observations necessarily include events occurring during recovery from exercise. Similarly, when human subjects are exercised on a bicycle ergometer, events concerning glucose metabolism have usually been studied only on completion of the exercise.

Increased muscle glucose uptake during or immediately after muscle exercise is at least in part due to increased plasma membrane GLUT-4, reported to be entirely in the membrane of T tubules; there is no increase in plasma membrane GLUT-1 (35). The increased plasma membrane GLUT-4 is by a factor of only ~2, occasionally more, sometimes less, small compared with the effect of insulin in vivo. As is the case for insulin stimulation, in most reports the degree of increase in skeletal muscle glucose uptake induced by exercise exceeds that in plasma membrane GLUT, suggesting that there may be an exercise effect on transport efficiency (72). It has been suggested that the discrepancy between increased glucose uptake and increased cell surface and T tubule GLUT-4 can be accounted for by the fact that GLUT-4 is a more efficient transporter than GLUT-1, but that argument is false. If increased glucose uptake is to be accounted for entirely by translocation of GLUT, the increase in GLUT-4 must actually be greater than that of glucose uptake, as long as the increase in surface GLUT-1 is less than that of glucose uptake, no matter how much more efficient GLUT-4 is than GLUT-1 (131).

All but one (50) of many studies report that combination of exercise and insulin increases both membrane GLUT-4 and glucose uptake. These increases have generally been found to be approximately additive or not quite additive (19), and, in one case, ~50% greater than purely additive, so that synergism was claimed (32). The bulk of these observations suggest that exercise stimulates translocation from a pool of GLUT-laden intracellular vesicles different from the pool stimulated by insulin. The suggestion is strong if maximum effects of insulin and exercise are additive, but not if the combined effects are submaximal. If the hypothesis is correct, one should find that exercise decreases GLUT from a different intracellular pool than that decreased by insulin. This has been reported to be the case (36). Consistent with, but not unequivocally supporting, the hypothesis is the observation that exercise increased glucose uptake in membrane (combined sarcolemma and T tubules) vesicles in obese Zucker rats (who do not respond to insulin) by at least as much as it does in matched lean Zucker rats (71). Two separate intracellular GLUT-4 pools have now been identified, one responding to insulin and the other to contraction (26).

How does exercise stimulate translocation of GLUT-4 to the T tubule? How does it increase glucose transport efficiency? These questions have several levels of answers. At the more immediate level, it is proposed, on
the basis of studies of isolated perfused rat hindlimb, that increased muscle blood flow, by increasing glucose delivery, adds to the effects of muscle contraction. At a more general level, more than 40 years ago, studies from Levine’s laboratory (Goldstein et al., 46a) showed, in eviscerated-nephrectomized dogs and rats, that hindlimb contractions against a load caused a generalized uptake of nonmetabolizable glucose analogs to the same extent as did insulin, even in dogs with low spinal cord section to eliminate neural pathways from the exercising muscles. This suggested strongly that exercising muscle produced a humoral factor that acted on all skeletal muscles, even those that were at rest during exercise of hindlimbs. A few years later, the same laboratory reported that, in parabiotic eviscerated-nephrectomized dogs, exercise by one dog increased sugar uptake by both dogs, increasing the likelihood that a humoral factor is released from exercising muscle (46). These intriguing observations have not been adequately pursued, and the mechanism by which exercise increases glucose uptake remains basically not understood. An observation taken to deny that a substance is released from exercising muscle to stimulate idle muscles to transport glucose comes from an isolated perfused rat hindlimb preparation in which there was no stimulation of glucose uptake in resting muscles perfused with a medium used previously in the exercise experiment (13). This report is less than crucial evidence against Levine’s proposal that exercise causes production or release into the circulation of a substance that increases glucose uptake; failure to obtain glucose uptake by the old perfusing medium may have been a dilution effect, or it may have been that the insulinomimetic factor does not arise in the exercised muscle, for example.

Exercise has been reported to increase sensitivity to insulin; that is, more glucose is transported for the same dose of insulin a few minutes after exercise or muscle contractions (see Ref. 45 for earlier references). It is reported that there is a factor in serum, probably a protein, that interacts with exercise (although not demonstrated to have been produced by exercise) in such a way as to increase glucose uptake by excised rat muscle in response to insulin, compared with the insulin response of the muscle when incubated in the absence of serum (45). With a regular program of heavy exercise, there is increased GLUT-4 mRNA and protein, and in response to insulin there is a greater translocation of GLUT-4 to the cell surface and greater glucose transport. Because the ratio of glucose uptake to cell surface GLUT-4 in response to insulin is about the same (both responses being increased by ~50%) before and after exercise training, it is suggested that the increase in insulin-induced glucose uptake after exercise training is “fully accounted for by the appearance of cell surface GLUT-4” (102); however, as pointed out earlier in discussion of whether translocation of GLUTs to the cell surface fully accounted for insulin-stimulated glucose uptake, the data are consistent with the hypothesis that insulin-stimulated glucose uptake is accounted for in part by an increase in GLUT density on the cell surface and in part by increased effectiveness of GLUT (131).

There are no reports of an acute effect of exercise on adipocyte glucose uptake and plasma membrane GLUT density, but exercise training (wheel-cage exercise-trained rats) produces increased GLUT-4 both in adipocyte microsomal fraction and in plasma membranes due to increased adipocyte GLUT-4 synthesis; there was no change in GLUT-1 (56), similar to the increases in skeletal muscle. If acute exercise does produce a humoral factor that stimulates skeletal muscle glucose uptake, it might also stimulate adipocyte glucose uptake.

HYPOXIA

It has been known at least since 1958 that hypoxia stimulates glucose uptake by skeletal muscle (100). Studies were resumed in 1991 with an important report from the laboratories of Holloszy and Klip (Cartee et al., 21), who have continued to play a major role in the area of skeletal muscle metabolism. Excised rat epitrochlearis muscles increased glucose uptake by 6-fold when N₂ was substituted for O₂ in the chamber. Anoxia increased GLUT-4 content of a plasma membrane fraction by 2.4-fold, far less than it increased glucose transport, again raising the question of whether there is also an anoxia-induced increase in GLUT efficiency. Maximum effects of insulin and anoxia were additive, whereas effects of anoxia and muscle contraction on glucose uptake were not additive, suggesting that the exercise and anoxia stimulate translocation of the same pool of intracellular vesicles containing GLUT-4 and that insulin stimulates a different pool. This suggestion is supported by observation on muscle strips obtained by biopsy from insulin-resistant obese or diabetic subjects in whom anoxia elicited the same glucose uptake response as seen in muscles from normal, lean subjects (7).

After 5 days of severe exercise training of rats, excised skeletal muscle has smaller responses to hypoxia with regard to both glucose uptake and GLUT-4 translocation (102). The authors propose that increased muscle glycogen content, which accumulates with exercise training, is responsible for decreased sensitivity to hypoxia.

SUMMARY AND PROJECTION

Glucose is an important substrate for oxidative consumption by nearly all cells; however, except for neurons, it is not the major substrate. Glucose is distributed in a heterogeneous concentration throughout extracellular fluid. Except for liver and kidney, although they produce glucose and release it into their effluent blood, arterial glucose concentration exceeds venous, and venous glucose concentration varies among organs and tissues depending on rate of glucose uptake and rate of blood flow. Among mammalian species, the ratio of glucose concentration in erythrocyte water to that in plasma water varies from 0 to 1. Erythrocyte glucose and glycogen serve to buffer plasma glucose.
concentration. Interstitial glucose concentration varies; possibly it is one-half that in arterial plasma water. Better and more rapidly responding methods for measuring interstitial fluid glucose concentration are needed.

All cell uptake and output of glucose are mediated only via specific glucose transporters, SGLT and GLUT. SGLTs transport glucose against a concentration gradient. This active transport is said to be fueled by coupling it to passive transport of Na^+, but although this conjecture has been repeated for decades, details of the coupling mechanism remain unknown. The family of transporters probably has varying affinities for glucose and transport glucose with varying efficiency. We do not know the details of the mechanism by which glucose passes between interstitial fluid and cytoplasm via a glucose transporter. Is the process one of iterative association and dissociation between a glucose molecule and the transporter through the thickness of the membrane? How many glucose molecules are bound to a single transporter at any instant? Without such knowledge, we lack the correct model by which to calculate transporter affinities. Although affinities have been reported as Michaelis-Menten $K_m$ values, we do not know that such calculations are correct.

With a CHO meal, there is accelerated gastric emptying, cycling of SGLT in small intestinal enterocytes to increase glucose absorption, with release of incretins to stimulate insulin release, hyperinsulinemia, recruitment of GLUT to cell surfaces of fat cells and skeletal and cardiac muscle with increased glucose uptake by these tissues, perhaps due also to activation of GLUT in the plasma membrane, and coordinated blocking of hepatic and renal glycogenolysis and gluconeogenesis. At the same time there may also be increased blood flow to skeletal muscle, increasing glucose delivery, and increased storage of glucose as glycogen in muscle, decreased lipolysis, and increased triglyceride storage in adipocytes. With chronic increase in dietary CHO there is increased synthesis of both SGLT and GLUT. CHO in the gut stimulates release of intestinal incretins that, in turn, stimulate release of insulin. As glucose is absorbed, circulating glucose concentration rises; there follows a complex hormonal interplay between insulin, which stimulates glucose uptake and tends to reduce circulating glucose concentration, and a number of other hormones that tend to raise it, either by opposing the action of insulin or by stimulating hepatic and renal glycogenolysis and gluconeogenesis. Our understanding of exactly how certain hormones oppose the action of insulin on glucose uptake is incomplete.

When insulin increases glucose uptake by adipose cells and skeletal and cardiac muscle, there is an increase in GLUT in the plasma membrane due to migration of intracellular vesicles to fuse with the plasma membrane. GLUTs are laced in the wall of these intracellular vesicles. There is an unresolved question as to whether this translocation of intracellular GLUT to the cell surface membrane accounts for all of insulin-stimulated glucose uptake. There are strong suggestions that the increase in cell surface GLUT is inadequate to account for observed glucose uptake. It is suggested that insulin also somehow increases GLUT effectiveness or turnover number so that a GLUT molecule transports more glucose molecules per unit time. We have some evidence, as yet incomplete, about the chain of signals by which insulin leads to translocation of GLUT-laden vesicles to the plasma membrane. If insulin also increases GLUT turnover number, that mechanism is quite unknown. I think of a possible mechanism as being related to one of the mechanisms by which ion channels are gated; a change in configuration of the transporter caused by some as-yet-identified mechanism, which may be, for example, an altered transmembrane electrical field, flips the transporter from an inactive or poorly active state to a more active state.

With exercise, glycogenolysis and gluconeogenesis are stimulated by a number of related factors, including particularly glucagon and catecholamines. Glucose uptake is increased. There is GLUT recruitment to the T tubular membrane in skeletal muscle from an intracellular pool distinct from that drawn on by insulin. Again, there is a possibility that GLUT intrinsic transport activity may be increased, and also that increased muscle blood flow may increase glucose delivery to previously underperfused fibers. Hypoxia has an effect similar to the acute effect of exercise, apparently drawing on the same GLUT pool. With sustained exercise training there is increased synthesis of GLUT in muscle and fat cells. There is a strong suggestion that exercise or muscle contraction produces a humoral factor that initiates GLUT recruitment in skeletal muscle, even in unstimulated muscles. It is not known whether the same acute effect occurs in adipocytes, but the effect of exercise training to increase the population of muscle GLUT, presumably by increased synthesis, is observed also in adipocytes. The stimulus observed with acute exercise may not be the same as that produced by sustained exercise training.

In vivo glucose uptake differs from in vitro in that 1) blood flow can determine the rate of glucose delivery to an organ or tissue, 2) artifacts occurring in tissue preparation that may affect such properties as cell surface GLUT density are avoided, 3) unknown and unrecognized factors not present in vitro may modify glucose uptake and output, and 4) in vivo experiments have their own artifacts.

Insulin can stimulate NO synthesis in arteriolae. It is possible that this is the mechanism by which insulin can increase blood flow, but the story is complicated by the fact that direct intra-arterial insulin infusion does not cause vasodilatation, at least not to the degree reported when insulin is administered in a fashion capable of lowering circulating glucose concentration. Nor is it proven that skeletal or cardiac muscle glucose uptake must increase simply because of increased blood flow; there is evidence that the two insulin effects are unrelated. What, then, is the function of insulin-induced vasodilatation?
Although some may argue otherwise, an ideal model of total body blood glucose kinetics and distribution has yet to be produced. Such a model ought to recognize the role of distribution of blood flow, heterogeneity of glucose uptake and, hence, heterogeneity of interstitial fluid and venous glucose concentrations, and mechanisms by which SGLT and GLUT transport glucose. The model might consist of a weighted number of parallel terms, perhaps each in the form of a modified Michaelis-Menten expression, to allow for the different affinities of the GLUT isoforms and for the fact that each GLUT is capable of transport in either direction. Weighting factors need to include distributions of transit times through blood, across capillaries, and through interstitial fluid to the GLUT surface.

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REFERENCES


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