Koopmans, Sietse J., Lawrence Mandarino, and Ralph A. DeFronzo. Time course of insulin action on tissue-specific intracellular glucose metabolism in normal rats. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E642–E650, 1998.—We investigated the time course of insulin action in conscious rats exposed to constant physiological hyperinsulinemia (–100 mU/l) while maintaining euglycemia (–100 mg/dl) for 0, 0.5, 2, 4, 8, or 12 h. [3-3H]glucose was infused to quantitate whole body glucose disposal (rate of disappearance, Rd), glycolysis (generation of \( \text{H_2O} \) in plasma), hepatic glucose production (HGP), and skeletal muscle and liver glycogen synthesis ([3-H]glucose incorporation into glycogen and time-dependent change in tissue glycogen concentration). The basal \( R_d \), which equals HGP, was 6.0 ± 0.3 mg·kg\(^{-1} \)·min\(^{-1} \). With increased duration of hyperinsulinemia from 0 to 0.5 to 2 to 4 h, \( R_d \) increased from 6.0 ± 0.3 to 21.0 ± 1.1 to 24.1 ± 1.5 to 26.6 ± 0.6 mg·kg\(^{-1} \)·min\(^{-1} \) (\( P < 0.05 \) for 2 and 4 h vs. 0.5 h). During the first 2 h the increase in \( R_d \) was explained by parallel increases in glycolysis and glycogen synthesis. From 2 to 4 h the further increase in \( R_d \) was entirely due to an increase in glycolysis without change in glycogen synthesis. From 4 to 8 to 12 h of hyperinsulinemia, \( R_d \) decreased by 19% from 26.6 ± 0.6 to 24.1 ± 1.1 to 21.6 ± 1.8 mg·kg\(^{-1} \)·min\(^{-1} \) (\( P < 0.05 \) for 8 h vs. 4 h and 12 h vs. 8 h). The progressive decline in \( R_d \) in the face of constant hyperinsulinemia, occurred despite a slight increase (8–14%) in glycolysis and was completely explained by a marked decrease (64%) in muscle glycogen synthesis. In contrast, liver glycogen synthesis increased fourfold, indicating an independent regulation of muscle and liver glycogen synthesis by long-term hyperinsulinemia. In the liver, during the entire 12-h period of insulin stimulation, the contribution of the direct (from glucose) and the indirect (from C-3 fragments) pathways to net glycogen formation remained constant at 77 ± 5 and 23 ± 5%, respectively. HGP remained suppressed throughout the 12-h period of hyperinsulinemia.

**IN RESPONSE TO A** physiological increment in the plasma insulin concentration there is a characteristic delay in the onset of insulin action, followed by a gradually increasing phase of enhanced glucose uptake that takes several hours to reach a plateau even though the plasma insulin concentration is unchanging (7, 9, 22). This time sequence of insulin action is best observed using the euglycemic insulin clamp, where plasma insulin and glucose concentrations are maintained constant, yet 2–4 h are required to achieve steady-state conditions for glucose utilization (7, 9, 22). This phenomenon has been referred to as the “self-amplifying effect” of insulin on insulin action (7, 9, 22). After 4–6 h, despite the presence of constant euglycemic hyperinsulinemia, there is progressive decline in glucose utilization, i.e., hyperinsulinemia appears to induce insulin resistance (8, 13). The mechanisms responsible for these phenomena are poorly understood. At the start of an insulin clamp, the lag phase between the attainment of constant plasma insulin levels and a steady-state rate of glucose disposal can be explained in part by the time required for plasma insulin to equilibrate with the remote compartments where insulin exerts its biological effects (14). However, because it has been shown that insulin is transported across vascular endothelial cells with a delay of 10 min (12) and it takes ~20 min before lymph insulin concentrations reach steady-state levels (33), other mechanisms must be responsible for the self-amplifying effect of insulin on whole body glucose utilization. Insulin has a time-dependent effect on glycolysis and glycogen synthesis, which is dependent on activation of key intracellular enzymes and transport steps, and these intracellular pathways for glucose metabolism are differently regulated in skeletal muscle and liver. Similar arguments can be made for the ability of chronic hyperinsulinemia to induce insulin resistance for whole body glucose utilization.

To investigate the processes responsible for the time-dependent effects of insulin on glucose disposal in vivo, we have studied the time course of insulin action on tissue-specific intracellular glucose metabolism in conscious normal rats. We used the hyperinsulinemic euglycemic clamp technique in combination with [3-3H]glucose infusion (17, 18, 29, 31) to quantitate whole body glucose uptake, glycolysis (appearance of \( \text{H_2O} \) in plasma), hepatic glucose production (HGP), and skeletal muscle and liver glycogen synthesis (incorporation of [3-H]glucose into glycogen and time-dependent increment in tissue glycogen concentrations).

**MATERIALS AND METHODS**

**Animals**

Eight groups (6–7 per group) of male Sprague-Dawley rats (300–350 g; Charles River, Wilmington, MA) were studied to determine the time-dependent action of insulin on intracellular glucose metabolism. Two groups of rats were studied in the basal state, i.e., no insulin infusion, one at 1000 (AM group), and the other at 2000 (PM group) to account for any circadian influence on basal glucose metabolism. Two groups of rats also were studied after 4 h of hyperinsulinemia, one at 1000 (AM group) and the other at 2000 (PM group) to account for any possible circadian influence on insulin-mediated glucose metabolism (20). The other four groups were used to...
study the effects of 0.5, 2, 8, and 12 h of hyperinsulinemia on glucose utilization; these insulin clamp studies were started at 0800. All rats were given free access to food and water, housed in individual cages in an air-controlled room, and subjected to a standard light (0600–1800)-dark (1800–0600) cycle. Four to six days before the insulin clamp experiments, rats were anesthetized with pentobarbital sodium (50 mg/kg body wt ip) and indwelling catheters were inserted into the right internal jugular vein and the left carotid artery. Both catheters were exteriorized through the skin at the back of the neck. Only rats that reached their preoperative weight within 4–6 days after surgery were studied. Three animals were excluded on the basis of this criterion.

Euglycemic Insulin Clamp Studies

After a 24-h fast, basal whole body glucose uptake (which equals HGP) was measured in conscious, unrestrained rats using [3-3H]glucose (DuPont-NEN, Boston, MA) infusion as previously described (11, 17, 18, 29, 31). [3-3H]Glucose was administered as a prime (4.7 µCi)-constant (0.15 µCi/min) infusion during the last 100 min of the 2-, 4-, 8-, and 12-h insulin clamp studies. Blood samples for determination of plasma tritiated glucose specific activity were obtained at 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, and 100 min after the start of [3-3H]Glucose. Because of the short time duration of the study, no [3-3H]Glucose was administered during the 0.5-h insulin clamp. Instead, in a separate group of rats \( n = 6 \), insulin and [3-3H]Glucose were infused for 100 min and blood samples were taken every 5 min to measure plasma glucose specific activity. We used the average of the time points \( t = 25, 30, \) and \( 35 \) min to calculate the 0.5-h time point. Steady-state conditions for plasma [3-3H]Glucose specific activity were reached within 30 min after initiation of [3-3H]Glucose infusion in all hyperinsulinemic study protocols. During the hyperinsulinemic euglycemic clamp studies, food was withheld from the animals and insulin was administered as a primed (104 mU·kg\(^{-1}\)·min\(^{-1}\))-continuous (6 mU·kg\(^{-1}\)·min\(^{-1}\)) infusion, and a variable infusion of a 25% glucose solution was started after initiation of [3-3H]glucose infusion. The total amount of glucose infused during both the basal and insulin infusion periods. During this steady-state period the rate of glucose appearance \( (Ra) \) equals the rate of glucose disappearance \( (Rd) \), and the glucose turnover rate was calculated by dividing the [3-3H]glucose infusion rate (dpm/min) by the steady-state plasma [3-3H]glucose specific activity (dpm/mg) (11, 17, 18, 29, 31). In the basal state, \( R_b \) equals the rate of HGP. In the insulin-stimulated state, \( R_s \) equals the rate of HGP plus the rate of exogenous glucose infusion. Therefore, HGP equals \( R_b \) minus exogenous glucose infusion. In the insulin-stimulated state, \( R_b \) equals the rate of whole body glucose uptake.

Whole body glycolysis and whole body glucose storage. \(^3\)H in the C-3 position of glucose is lost selectively to \(^2\)H\(_2\)O during glycolysis. Therefore, plasma tritiated counts are present either as \(^2\)H\(_2\)O or as [3-3H]Glucose. Rates of whole body glycolysis were determined from the increment per unit time in \(^2\)H\(_2\)O (dpm·1·min\(^{-1}\)) multiplied by the total body water mass and divided by the [3-3H]glucose specific activity (dpm/mg) (11, 17, 18, 29, 31). Plasma \(^2\)H\(_2\)O is assumed to be 93% of the
Table 1. Body weight and plasma hormone and substrate concentrations during euglycemic hyperinsulinemic clamps

<table>
<thead>
<tr>
<th>Duration of Hyperinsulinemia, h</th>
<th>0</th>
<th>0.5</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>318 ± 6</td>
<td>314 ± 6</td>
<td>321 ± 8</td>
<td>313 ± 5</td>
<td>318 ± 7</td>
<td>328 ± 3</td>
</tr>
<tr>
<td>Insulin, mU/l</td>
<td>16 ± 1</td>
<td>125 ± 8</td>
<td>110 ± 7</td>
<td>118 ± 11</td>
<td>106 ± 13</td>
<td>129 ± 9</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>104 ± 4</td>
<td>103 ± 9</td>
<td>100 ± 6</td>
<td>104 ± 6</td>
<td>107 ± 6</td>
<td>104 ± 4</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>1.5 ± 0.1</td>
<td>0.9 ± 0.1*</td>
<td>0.8 ± 0.1*</td>
<td>0.5 ± 0.1†</td>
<td>0.5 ± 0.1†</td>
<td>0.5 ± 0.1†</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>0.6 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.5 ± 0.4‡</td>
<td>1.6 ± 0.3‡</td>
<td>1.6 ± 0.1‡</td>
<td>1.6 ± 0.1‡</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>51 ± 8</td>
<td>&lt;25</td>
<td>&lt;25</td>
<td>&lt;25</td>
<td>&lt;25</td>
<td>&lt;25</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6-7 rats/group. FFA, free fatty acid. *P < 0.05 vs. 0 h. †P < 0.01–0.05 vs. 0, 0.5, and 2 h. ‡P < 0.05 vs. 0 and 0.5 h.
observed during the first 2 h of hyperinsulinemia ($r = -0.50, P < 0.025$). Plasma glucagon concentration was maximally suppressed by insulin within 30 min after the start of euglycemic hyperinsulinemia.

**Whole Body Glucose Disposal, Glycolysis, Glucose Storage, and HGP**

The time course of insulin action on whole body glucose disposal ($R_d$), whole body glycolysis, and glucose storage (or whole body nonglycolytic glucose disposal) is shown in Fig. 1. Whole body glucose storage (nonglycolytic glucose disposal) primarily represents glycogen synthesis (29) plus a very small amount of glucose disposal in lipid (3). Basal $R_d$, 6.0 ± 0.3 mg·kg⁻¹·min⁻¹, increased 3.5-fold to 21.0 ± 1.1 mg·kg⁻¹·min⁻¹ within the first 0.5 h of hyperinsulinemia. From 0.5 to 2 to 4 h, $R_d$ rose further to 24.1 ± 1.5 and 26.6 ± 0.6 mg·kg⁻¹·min⁻¹, respectively ($P < 0.05$ for 2 and 4 h vs. 0.5 h). Thereafter, $R_d$ declined progressively by 19% to 24.1 ± 1.1 and 21.6 ± 1.8 mg·kg⁻¹·min⁻¹ at 8 and 12 h ($P < 0.05$ for 8 h vs. 4 h and 12 h vs. 4 h). During the 0- to 2-h time period the increases in whole body glycolysis (Fig. 1B) and whole body glucose storage (Fig. 1C) closely paralleled the increase in whole body $R_d$. However, from 2 to 4 h a clear-cut dissociation between the rates of whole body glycolysis and whole body glucose storage was observed. From 2 to 4 h of hyperinsulinemia, the increment in $R_d$ ($\Delta = 2.5$ mg·kg⁻¹·min⁻¹) was paralleled by a nearly identical increase ($\Delta = 2.3$ mg·kg⁻¹·min⁻¹) in glycolysis while, during the same time period, no change in glucose storage ($\Delta = 0.2$ mg·kg⁻¹·min⁻¹) was observed.

From 4 to 8 to 12 h of hyperinsulinemia, whole body $R_d$ decreased by 19%, $P < 0.05$. The induction of whole body resistance by physiological hyperinsulinemia occurred despite a slight increase ($\Delta = 1.2$ mg·kg⁻¹·min⁻¹ at 12 h) in whole body glycolysis and was completely explained by a marked decrease ($\Delta = 6.1$ mg·kg⁻¹·min⁻¹ at 12 h) in whole body glucose storage. Basal HGP, 6.0 ± 0.3 mg·kg⁻¹·min⁻¹, decreased to 1.2 ± 0.9 mg·kg⁻¹·min⁻¹ within 0.5 h after the start of insulin and remained suppressed throughout the 12-h period of hyperinsulinemia.

**Muscle and Liver Glycogen Synthesis**

In rectus abdominal and psoas muscles, glycogen synthetic rates were calculated by two independent means: 1) from the incorporation of [3-3H]glucose counts into muscle glycogen and 2) from the time-dependent increment in cold muscle glycogen concentration. Both methods yielded identical results. The concentrations of glycogen in muscle after 0, 0.5, 2, 4, 8, and 12 h of hyperinsulinemia were 0.40 ± 0.02, 0.57 ± 0.02, 0.73 ± 0.04, 0.93 ± 0.04, 1.17 ± 0.06, and 1.32 ± 0.05%, respectively. Because the muscle fiber types in abdominlus rectus and psoas muscles are representative of those in the whole body skeletal muscle (2, 26), we have calculated the whole body skeletal muscle glycogen synthetic rate, assuming that skeletal muscle represents 40% of body weight (Fig. 2A). From the qualitative standpoint, the rate of whole body skeletal muscle glycogen synthesis (calculated either from the incorporation of [3-3H]glucose into glycogen or the increment in cold muscle glycogen concentration; Fig. 2A) closely paralleled the rate of whole body glucose storage (calculated as the difference between total $R_d$ and the rate of glycolysis; Fig. 1C). From the quantitative standpoint, however, the directly measured rate of skeletal muscle glycogen synthesis by [3-3H]glucose incorporation into glycogen was 10.2 ± 3.3 mg·kg⁻¹·min⁻¹ at 12 h.

![Fig. 1. Time-dependent effect of physiological euglycemic hyperinsulinemia on whole body glucose uptake (A: rate of disappearance ($R_d$)), whole body glycolysis (B), whole body glucose storage (C: $R_d$ – glycolysis), and hepatic glucose production (D) in 6–7 conscious, unrestrained rats per time point. *P < 0.05 vs. each preceding time point.](http://ajpendo.physiology.org/ Downloaded from http://ajpendo.physiology.org/ by [user](http://ajpendo.physiology.org/) on July 11, 2017)
glycogen synthesis (Fig. 2A) was ~33% less than the rate of whole body glucose storage (Fig. 1C). The concentrations of glycogen in liver after 0, 0.5, 2, 4, 8, and 12 h of hyperinsulinemia were 0.21 ± 0.01, 0.20 ± 0.02, 0.34 ± 0.09, 0.62 ± 0.11, 1.22 ± 0.25, and 3.11 ± 0.48%, respectively. For rectus and psoas muscles, as well as for liver, we also examined the correlation between tissue glycogen concentration and glycogen synthetic rate. Rather than pooling the data, as presented in Fig. 3, we carried out the analysis using the following time intervals: 0.5 to 4 h, 4 to 12 h, and 8 to 12 h of hyperinsulinemia. None of the individual time intervals showed a significant correlation (all \( P > 0.10 \)) between glycogen concentration and glycogen synthetic rate in the two muscle groups. This suggests that an increase in muscle glycogen concentration is not responsible for the time-related decrease in muscle glycogen synthetic rate. The liver showed a positive correlation at all time frames (\( r = 0.75, P < 0.005; r = 0.877, P < 0.0001; r = 0.857, P < 0.005 \), respectively). The rate of whole body liver glycogen synthesis, calculated from the incorporation of \([3-3H]\)glucose into glycogen, was smaller (by 23 ± 5%, \( P < 0.05 \)) than the rate measured from the increment in cold glycogen concentration (Fig. 2B). Thirty minutes of euglycemic hyperinsulinemia had no stimulatory effect on glycogen synthesis. Thereafter the rate of liver glycogen synthesis increased linearly at the rate of 1.0 ± 0.3 mg·kg\(^{-1}\)·min\(^{-1}\) from 0.5 to 8 h. From 8 to 12 h the rate of liver glycogen synthesis increased threefold to 3.2 ± 0.9 mg·kg\(^{-1}\)·min\(^{-1}\) (\( P < 0.05 \) vs. 0.5- to 8-h period; Fig. 2B). The contribution of the direct (from glucose) and the indirect (C-3 fragments) pathways to net glycogen synthesis remained constant at 77 ± 5 and 23 ± 5%, respectively, at all time intervals from 2 to 12 h. During the initial 4 h of hyperinsulinemia, liver glycogen synthesis (−1 mg·kg\(^{-1}\)·min\(^{-1}\); Fig. 2B) and muscle glycogen synthesis (−8 mg·kg\(^{-1}\)·min\(^{-1}\); Fig. 2A) accounted for ~75% of whole body glucose storage (Fig. 1C). Lipid synthesis accounted for an additional 1 mg·kg\(^{-1}\)·min\(^{-1}\) (see below), leaving unaccounted for about 15% of whole body glucose storage. This later 15% was accounted for by tritiated labeled water-soluble compounds in the tissue. Tissue glycogen concentration has been suggested to be an important determinant of the rate of glycogen synthesis. In both abdominus rectus (\( r = -0.257, P > 0.25 \); Fig. 3A) and psoas (\( r = -0.179, P > 0.25 \); Fig. 3B) muscles, the tissue glycogen concentration showed no correlation with the tissue glycogen synthetic rate, as determined by the incorporation of \([3-3H]\)glucose counts in glycogen. In contrast, liver glycogen concentration showed a significant positive correlation with the liver glycogen synthetic rate (\( r = 0.889, P < 0.0001 \); Fig. 3C).

![Fig. 2. Time-dependent effect of physiological euglycemic hyperinsulinemia on whole body skeletal muscle glycogen synthesis (A) and whole body liver glycogen synthesis (B) in 6–7 conscious, unrestrained rats per time point. Mean values of rectus abdominus and psoas muscles were taken to be representative of whole body skeletal muscle mass, and we assumed that skeletal muscle represented 40% of whole body weight. For whole body liver glycogen synthesis we assumed that liver was 4% of whole body weight. *\( P < 0.05 \) vs. each preceding time point.](http://ajpendo.physiology.org/)

![Fig. 3. Correlation among rectus abdominus muscle (A), psoas muscle (B), and liver (C) glycogen concentrations (in mg glucosyl units per g tissue) and corresponding insulin-stimulated tissue glycogen synthetic rate as determined by incorporation of \([3-3H]\)glucose counts into glycogen. Each symbol represents an individual rat (n = 25 rats).](http://ajpendo.physiology.org/)
Skeletal Muscle, Liver, and Adipose Tissue Lipid Synthesis

Whole body skeletal muscle, liver, and adipose tissue lipid synthesis was estimated from the incorporation of \([3-^{3}H]glucose\) counts into the lipid fraction of psoas rectus abdominus muscle, liver, and adipose tissue with the assumption that these organs represent 40, 4, and 10%, respectively, of the rat's body weight. In muscle, lipid synthesis quickly reached a plateau of 0.6 ± 0.1 mg·kg\(^{-1}\)·min\(^{-1}\) by 2 h (Fig. 4A). In contrast, liver lipid synthesis (Fig. 4B) rose slowly and progressively during the first 8 h but accounted for only 0.1–0.2 mg·kg\(^{-1}\)·min\(^{-1}\). From 8 to 12 h the rate of liver lipid synthesis rose more steeply, reaching a value of 0.5 ± 0.1 mg·kg\(^{-1}\)·min\(^{-1}\). Adipose lipid synthesis was quite small, 0.3–0.4 mg·kg\(^{-1}\)·min\(^{-1}\), and remained constant throughout the 12-h period of hyperinsulinemia (Fig. 4C).

After 12 h of hyperinsulinemia, lipid synthesis (−1.1 mg·kg\(^{-1}\)·min\(^{-1}\), muscle glycogen synthesis (−2.5 mg·kg\(^{-1}\)·min\(^{-1}\)), and liver glycogen synthesis (−3.2 mg·kg\(^{-1}\)·min\(^{-1}\)) could account for whole body glucose storage (Fig. 4C).

**Discussion**

An increase or decrease in insulin sensitivity could result from an alteration in the insulin receptor-signal transduction system, glucose transport, glucose phosphorylation, and/or from a change in the activity of one or more enzymes in the two major intracellular metabolic pathways of glucose disposal, i.e., glycogen synthesis or glycogenolysis. If an early alteration in insulin action, i.e., insulin receptor-signal transduction or glucose transport-phosphorylation, were responsible for the time-related change in insulin action, one would expect the rates of glycogen synthesis and glycogenolysis to change in parallel. During the initial 2 h after the start of insulin, the self-amplifying effect of the hormone is accounted for by parallel increases in glucose storage (Fig. 1C) and glycogenolysis (Fig. 1B). This observation is consistent with the stimulation of an early step in insulin action or parallel and independent stimulatory effects of insulin on glucose storage and glycologly.

It should be noted that during the same time period hepatic glycogen synthesis increased exponentially (Fig. 2B), indicating an independent and opposite regulation of muscle and liver glycogen synthesis by chronic hyperinsulinemia. Hepatic glycogen formation occurs through a direct (via glucose) and an indirect (C-3 fragments like lactate) pathway (34). Our data exclude the possibility that the observed exponential increase...
in hepatic glycogen formation is caused by a major shift in the contribution of the direct (77%) vs. the indirect (23%) pathway to glycogenesis. It is interesting to compare the mechanisms that control glycogen synthesis in liver and muscle. In liver, hyperglycemia is the primary stimulus for glycogen synthesis (6, 11), whereas in muscle, tissue glycogen concentration (25) and plasma insulin concentration (23, 29) are the major regulators of glycogen synthesis. Concerning the former we now show that long-term (8–12 h) hyperinsulinemia, without any change in the blood glucose concentration, can markedly increase liver glycogen synthesis even in the presence of a threefold increase in liver glycogen concentration. This stimulatory effect of insulin on hepatic glycogen synthesis has not previously been appreciated with short-term (2 h) hyperinsulinemia in vivo (7). The mechanism(s) responsible for the time-dependent increase in hepatic glycogen formation during euglycemic hyperinsulinemia currently is under investigation and may involve 1) stimulation of hepatic glycogen synthase activity (23), 2) increased GLUT-2 transporter expression (27), 3) increased glucokinase expression (15), or 4) liver cell swelling (1). In skeletal muscle, a negative feedback mechanism between tissue glycogen concentration and the rate of glycogen synthesis has been described (25). The upper limit of muscle glycogen concentration is tightly controlled at ~1% or 10 mg glucosyl units per gram muscle (5, 19). In the present study we did not observe a significant negative correlation between the muscle glycogen concentration and the rate of glycogen synthesis. This may be explained by the observation that from 0 to 4 h of hyperinsulinemia the muscle glycogen concentration in our rats was below 1% (0.93 ± 0.04%) due to several factors including the low physiological hyperinsulinemic stimulus employed during the insulin clamp studies, the maintenance of euglycemia, and the 24-h period of fasting before the insulin clamp was performed. These relatively low muscle glycogen levels could have prevented activation of the negative feedback principle whereby the muscle glycogen concentration inhibits its own synthesis (5, 19, 25). After 8 and 12 h of hyperinsulinemia the muscle glycogen concentrations rose to 1.17 ± 0.06 and 1.32 ± 0.05%, respectively. At such muscle glycogen concentrations one should have expected to observe a negative feedback inhibition of insulin-mediated glucose disposal. However, we failed to observe any correlation between either the muscle glycogen concentration or the rate of glycogen synthesis in 25 rats (Fig. 3, A and B). Other investigators, including Vardanis and Hudson (32), also have failed to demonstrate any relationship between muscle glycogen concentration and the activity of glycogen synthase phosphatase or glycogen biosynthesis. The hyperinsulinemia-induced desensitization of skeletal muscle glycogen synthesis described in our study seems therefore to involve a mechanism(s) other than negative feedback by increased muscle glycogen concentrations. Our results also raise questions about the importance of this negative feedback mechanism in the regulation of glycogen synthesis under day-to-day physiological conditions.

The economy of whole body glucose metabolism is depicted in Table 2. After 4 h of physiological euglycemic hyperinsulinemia, whole body glucose disposal was maximal (26.6 mg·kg⁻¹·min⁻¹) and was approximately equally divided between glycolysis (i.e., glucose oxidation plus anaerobic glycolysis) and glucose storage (i.e., nonglycolytic glucose disposal). Muscle glycogen synthesis, liver glycogen formation, and glucose incorporation into lipids accounted for 7.0 (57%), 0.9 (7%), and 1.0 (8%) mg·kg⁻¹·min⁻¹, respectively, of whole body glucose storage. As previously noted (3), adipose tissue accounted for the disposal of about 1% of the infused glucose load, and the formation of fatty acids and lipid glycerol in muscle, liver, and adipose tissue was responsible for only 1.0 mg·kg⁻¹·min⁻¹ (8%) of whole body glucose storage. After 12 h of hyperinsulinemia whole body glucose uptake declined by 19%, and this was entirely due to a decrease in whole body glucose storage (Table 2). After 12 h liver glycogen synthesis increased threefold to 3.2 mg·kg⁻¹·min⁻¹ and accounted for 52% of whole body glucose storage. Lipid synthesis, 1.1 mg·kg⁻¹·min⁻¹, remained unchanged after 12 h of hyperinsulinemia but was responsible for a greater percentage (18%) of whole body glucose storage. The most dramatic effect of chronic hyperinsulinemia was the 64% reduction in muscle glycogen synthesis (7.0 to 2.5 mg·kg⁻¹·min⁻¹).

The physiological purpose of the differential regulation of muscle and liver glycogen synthesis by insulin may be as follows. Once hyperinsulinemia starts (i.e., with a meal), glycogen is first stored in muscle, where it subsequently is broken down and used locally for muscular activity. After the muscle glycogen storage depot is filled, if glucose supply continues, the liver is filled with glycogen and this serves as a buffer during the fasting state. After the exposure to insulin, glycolysis increases and remains activated without any desensitization. By maintaining glycolytic flux and glucose oxidation, fat oxidation is inhibited (Randle cycle) and this spares lipid stores.

A time-dependent change in insulin-mediated glucose uptake can be due to a change in the concentrations of circulating hormones and metabolites that antagonize insulin action or to circadian rhythmicity (20). We previously have shown that, under similar

<table>
<thead>
<tr>
<th>Table 2. Time-related change in whole body glucose metabolism</th>
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<tbody>
<tr>
<td><strong>Hours</strong></td>
</tr>
<tr>
<td>Whole body glucose uptake (R_g)</td>
</tr>
<tr>
<td>Whole body glycogenesis (Gly)</td>
</tr>
<tr>
<td>Whole body glucose storage (R_g – Gly)</td>
</tr>
<tr>
<td>Whole body muscle glycogen synthesis</td>
</tr>
<tr>
<td>Liver glycogen synthesis</td>
</tr>
<tr>
<td>Lipid synthesis</td>
</tr>
<tr>
<td>Skeletal muscle</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Adipose</td>
</tr>
</tbody>
</table>

Values are mg·kg⁻¹·min⁻¹; n = 6–7 rats/group. R_g, rate of glucose uptake; Gly, glycolysis.
conditions of chronic euglycemic hyperinsulinemia, plasma epinephrine and corticosteroid levels do not change significantly from baseline (16). In the present study we demonstrate that plasma glucagon levels are promptly suppressed by insulin and remain at undetectable levels throughout the study for up to 12 h. An increase in plasma FFA concentration and FFA oxidation (Randle cycle) (28) seems an unlikely cause of the insulin resistance because circulating FFA levels remained maximally suppressed from 4 to 12 h. The possible influence of circadian rhythmicty on basal and insulin-mediated glucose metabolism was investigated by studying rats at 1000 and at 2000. Under basal conditions at 2000, plasma glucose and insulin levels were significantly higher compared with 1000, suggesting the development of “basal” insulin resistance. However, when plasma insulin levels were raised to 100 mU/l, insulin-mediated glucose disposal was similar at 1000 and 2000. Thus the observed decline in whole body glucose disposal and glucose storage cannot be accounted for by a circadian change in insulin sensitivity.

The hexosamine pathway has been shown to play an important role in the hyperglycemia-induced development of insulin resistance. According to this hypothesis, elevated plasma glucose levels, by mass action, increase the flux of glucose into the muscle cells. This causes an increase in glucosamine synthesis, which in turn desensitizes glucose transport (24). It is possible that an increased flux of glucose into muscle cells, driven by hyperinsulinemia rather than by hyperglycemia, results in a comparable increase in glucosamine synthesis. Whether this pathway is involved in the hyperinsulinemia-induced insulin resistance for whole body muscle uptake observed in the present study remains to be established. Two recently published papers have presented equivocal findings concerning the effect of glucosamine infusion on in vivo insulin action (10, 30). As described by Giaccari et al. (10), desensitization of glucose transport by glucosamine caused a similar reduction in glycolysis and glycogen synthesis. Such an observation would not be compatible with our data. On the other hand, Rossetti et al. (30) showed that glucosamine infusion caused a greater reduction in insulin-stimulated glycogen synthesis compared with glycolysis, which was less affected. These later results (30) more closely resemble the pattern of hyperinsulinemia-induced insulin resistance described in the present study. This issue needs further study.

The present observations may have pathophysiologically relevance to the development of glucose intolerance in non-insulin-dependent diabetes mellitus (NIDDM) and obesity. Both of these disorders are characterized by hyperinsulinemia and insulin resistance, primarily involving the glycogen synthetic pathway in muscle. The most widely accepted thesis is that the insulin resistance is primary and that the hyperinsulinemia represents a compensatory response to offset the reduction in insulin action. The present results demonstrate that primary hyperinsulinemia can induce insulin resistance involving the muscle glycogen synthetic pathway.

It is commonly thought that hyperinsulinemia in NIDDM and obesity represents a compensatory response to the defect in insulin action. However, our results indicate that hyperinsulinemia per se can induce insulin resistance. Therefore, in both NIDDM and obesity, hyperinsulinemia also can be viewed as a self-perpetuating cause of insulin resistance. A more speculative hypothesis is suggested by the data of Lillioja et al. (21). In Pima Indians for any severity of insulin resistance the amount of insulin that is secreted by the pancreas is increased by 50% compared with Caucasians. This raises the intriguing possibility that the primary disturbance leading to NIDDM is enhanced insulin secretion and that the insulin resistance occurs secondarily to the sustained hyperinsulinemia.

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