Hepatic Overexpression of Insulin-like Growth Factor-II in Adulthood Increases Basal and Insulin-stimulated Glucose Disposal in Conscious Mice*

Luciano Rossetti‡§, Nir Barzilai‖, Wei Chen, Thomas Harris, Deyun Yang, and Charles E. Rogler‡

From the Division of Endocrinology, Diabetes Research and Training Center and the Liver Research Center, Albert Einstein College of Medicine, Bronx, New York 10461

The physiological role of circulating insulin-like growth factor-II (IGF-II) in adult humans is poorly understood. We recently generated an IGF-II transgenic murine model of persistent IGF-II production (plasma IGF-II ~30-fold increased above normal) through overexpression of the transgene driven by the major urinary protein promoter (Rinderknecht, E., and Humble, R. E. (1978) J. Biol. Chem. 269, 13779–13784). To determine whether in vivo insulin action is improved in these transgenic mice, we performed euglycemic insulin (18 milliunits/kg/min) clamp studies in conscious IGF-II transgenic and in age- and weight-matched control mice. Plasma glucose and insulin concentrations were significantly lower in the IGF-II transgenic compared with control mice (1978). The physiological role of circulating insulin-like growth factors I and II is poorly understood. We recently generated an IGF-II transgenic murine model of persistent IGF-II production (plasma IGF-II ~30-fold increased above normal) through overexpression of the transgene driven by the major urinary protein promoter (Rinderknecht, E., and Humble, R. E. (1978) J. Biol. Chem. 269, 13779–13784). To determine whether in vivo insulin action is improved in these transgenic mice, we performed euglycemic insulin (18 milliunits/kg/min) clamp studies in conscious IGF-II transgenic and in age- and weight-matched control mice. Plasma glucose and insulin concentrations were significantly lower in the IGF-II transgenic compared with control mice. Despite decreased plasma glucose concentration, basal hepatic glucose production (HGP) and glucose clearance were increased.

During the insulin clamp studies in IGF-II transgenic mice compared with control mice (a) the rates of glucose infusion and glucose uptake were increased by ~65 and ~55%, respectively; (b) glycolysis was increased by ~12% while glycogen synthesis was ~2-fold higher; (c) while the suppression of plasma free fatty acid was similar, the increment in plasma lactate concentration was significantly higher; (d) although HGP was similarly inhibited by insulin, phosphoenolpyruvate gluconeogenesis was enhanced and accounted for a larger portion of HGP (64% versus ~40% in control mice).

Our data suggest that the persistence of circulating IGF-II in adult mice to levels commonly observed in adult humans (50–70 nM) causes a marked improvement in peripheral (skeletal muscle) insulin action, which is not due to changes in body composition. These results suggest that circulating IGF-II may exert a regulatory role on insulin sensitivity and body composition in humans.

Insulin-like growth factors I and II are structurally related to proinsulin (1, 2) and exert growth promoting (3) and metabolic effects (4). In rodents, IGF-II plays an important role during fetal development (5, 6), while its gene expression and circulating concentrations are virtually suppressed postnatally (7). Conversely, elevated plasma IGF-II concentrations have been reported in adult humans (8) with only a slight decline with aging (9). IGF-I levels tend to progressively decrease in middle-aged and old humans (8, 9). Recent observations from several laboratories have spurred interest in the potential physiologic significance of circulating IGFs in the alterations in body composition and insulin sensitivity of human aging (10, 11). These observations suggest that IGF-II may have a metabolic role in humans.

To examine the potential metabolic function of IGF-II in adulthood, we generated transgenic mice, which overexpress the prepro IGF-II transgene in the liver starting at 3–5 weeks of age (12). These transgenic mice maintained elevated plasma IGF-II concentrations throughout their lives, in the range commonly observed in young human subjects (8, 12). Compared with control mice, IGF-II transgenic mice had lower plasma glucose and insulin concentrations and gained less weight between 4 and 18 months of age (12). The lower body weight was largely due to a marked decrease in fat mass (12). The metabolic impact of the persistence of high IGF-II levels in adult mice may be interpreted in view of our current understanding of IGFs action in vivo. IGF-II has been shown to stimulate glucose disposal during short term infusions in rodents (13) and humans (14). Yet, the effect of chronic elevations in the circulating IGF-II concentrations on glucose fluxes have not been examined. Although IGF-II binds with high affinity to the IGF-II/mannose 6-phosphate receptors, there is strong evidence that its growth promoting and metabolic effects are not mediated via this interaction (15). It is likely that IGF-II exerts its action acting through the IGF-I and/or the insulin receptor depending on the target tissue (15, 16). In this regard, it is of interest to note that in rodents, IGF-I has differential effects on intermediate metabolism compared with insulin, with more potent effects on protein metabolism and modest effects on lipid metabolism (17).

Since our previous observation of changes in body composition and plasma glucose concentrations suggested that IGF-II transgenic mice have specific changes in whole body glucose metabolism, we examined tissue sensitivity to insulin and basal glucose kinetics in transgenic and control mice using the

*This work was supported by grants from the National Institutes of Health (R029-DK 45024 and R01-DK 48321, R01-CA 56076, and DK 41296) and the Core Laboratories of the Albert Einstein Diabetes Research and Training Center (DK 20541). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Recipients of Career Scientist Awards from the Irma T. Hirschl Trust.
§ To whom correspondence should be addressed: Div. of Endocrinology, Dept. of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461. Tel.: 718-430-4215; Fax: 718-828-6988; E-mail: Rossetti@aeom.yu.edu.
‖ Recipient of a postdoctoral fellowship from the J. Greene Diabetes Foundation.
¶ Recipient of a postdoctoral fellowship from GI Training Grant T32 DK 07218.

The abbreviations used are: IGF-II, insulin-like growth factor-II; MUP, major urinary protein; HGP, hepatic glucose production; PEP, phosphoenolpyruvate; TGO, total glucose output; GC, glucose cycling; GH, growth hormone.
Insulin clamp technique in combination with tracer infusions. In order to estimate the impact of changes in body composition on insulin sensitivity, we also compared the IGF-II transgenic mice to a group of younger negative control mice matched for body weight. IGF-II transgenic mice had a marked increase in both basal and insulin-stimulated glucose uptake and glycogen synthesis, increased basal HGP, and increased contribution of gluconeogenesis to HGP during the insulin clamp studies. Since the above metabolic effects could not be ascribed to the associated changes in body composition, it is likely that they are the direct consequence of the elevated IGF-II levels.

**EXPERIMENTAL PROCEDURES**

Animals—The genetic background of mice used in this study was initially 75% C57BL/6, 25% CBA chromosomes (12). MUP IGF-II founder mice were bred with C57Bl/6 mice to produce the F2 generation. The homozygous MUP IGF-II and the negative control mice used in the present studies were obtained by interbreeding F2 generation littermates that were either positive or negative for the transgene (12). Nine male age-matched negative controls (group I; 8–10 months old), five male weight-matched negative controls (group II; 4–6 months old), and seven male homozygous MUP IGF-II mice (group III; 8–10 months old) received euglycemic insulin clamp studies (see below). All mice were anesthetized with intraperitoneal ketamine (100 mg/kg) and xylazine (5 mg/kg) given by rapid intraperitoneal injection and an indwelling catheter was inserted into the right internal jugular vein, as described previously in rats (18–20). Mice were studied 4–6 days postsurgery.

Euglycemic Clamp Studies—Studies were performed in awake, unrestrained, chronically catheterized mice using the euglycemic clamp technique in combination with [U-14C] lactate and [3-H] glucose infusions as described previously (21–23). Food was removed for 6 h before the in vivo studies. Briefly, a prime-continuous infusion of regular insulin (18 mU/kg/min) was administered, and a variable infusion of a 25% glucose solution was started at time zero and periodically adjusted to clamp the plasma glucose concentration at ~6.5 mm. The total volume was 170 ml and included an 80-min basal period for assessment of basal turnover rates and a 90-min euglycemic clamp period. 80 min before starting the insulin infusion, a prime-continuous infusion of high performance liquid chromatography-purified [3-H] glucose (DuPont NEN; 10 μCi bolus, 0.1 μCi/min) was initiated and maintained throughout the remainder of the study. [U-14C] lactate (5 μCi bolus, 0.25 μCi/min) was infused during the last 30 min of the study. Plasma samples for determination of [3-H] glucose specific activity were obtained from the tail vein at 40, 60, 70, and 80 min during the basal period and at 40, 60, 70, 80, and 90 min during the clamp period. Steady state conditions for the plasma glucose concentration and specific activity were achieved within 40 min in both the basal and clamp periods of the studies. Plasma samples for determination of plasma insulin concentrations (G) were obtained at time ~30, 0, 40, 60, 90 min during the study. The total volume of blood withdrawn was ~0.9 ml/study; to prevent volume depletion and anemia, a solution (1:1 (v/v)) of ~1.2 ml of fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 units/ml) was infused. All determinations were also performed on portal vein blood obtained at the end of the experiment. At the end of the insulin infusion, mice were anesthetized (pentobarbital 60 mg/kg body weight, intravenously), the abdomen was quickly opened, portal vein blood was obtained, and the liver was freeze-clamped in situ with aluminum tongs precooled in liquid nitrogen. The time from the injection of the anesthetic until freeze-clamping of the liver was less than 45 s. All tissue samples were stored at -80°C until analysis.

Body Composition—Lean body mass and fat mass were calculated from the whole body volume of distribution of water as described previously (12). The latter was derived from the steady-state concentration of [3-H] H2O in plasma water following the bolus injection of tritiated water. Briefly, 1 μCi of [3-H] H2O (DuPont NEN) was injected intraperitoneally, and plasma samples were collected at 60 min intervals between 1 and 3 h thereafter. Steady state for [3-H] H2O specific activity in rodents was achieved within 30–45 min.

The study protocol was reviewed and approved by the Institutional Animal Care and Use Committees of the Albert Einstein College of Medicine.

**Analytical Procedures**—Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Inc., Palo Alto, CA) and plasma insulin by radioimmunoassay using rat and porcine insulin standards. Plasma [3-H] glucose radioactivity was measured in duplicate on the supernatants of Ba(OH)2 and ZnSO4 precipitates (Somogyi procedure) for plasma samples (25 μl) after evaporation to dryness to eliminate tritiated water. The rates of glycolysis were estimated as described previously (24). Briefly, plasma-tritiated water specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation. Because tritium is lost to water during glycolysis, it can be assumed that plasma tritium is present either in tritiated water or [3-H] glucose. Uridine diphosphoglucose (UDP-Glc), uridine diphosphogalactose (UDP-Gal), and phosphoenolpyruvate (PEP) concentrations and specific activities in the liver were obtained through two sequential chromatographic separations, as reported previously (21, 22, 23). Muscle glycogen concentration and glycogen synthase activity and the hepatic activities of glycogenase and glucose-6-phosphatase were determined as described previously (19, 21, 22, 23). Aliquots of the tissue homogenate (100 μl) were also employed to determine the amount of tritium label in glycogen. Glycogen was precipitated by washing in 10 volumes of absolute ethanol and by incubating for 1 h at -20°C. The procedure was repeated 3 times, and then the precipitate was collected, dried down, and dissolved in water before scintillation counting. Differences between groups were determined by analysis of variance.

**RESULTS**—In the present manuscript, the term total glucose output (TGO) is intended as total in vivo flux through glucose-6-phosphatase as measured by [2-H]glucose turnover, and the term hepatic glucose production (HGP) is intended as total glucose output minus the contribution of plasma glucose to hepatic glucose-6-phosphatase dephosphorylation to glucose as measured by [3-H] glucose turnover. Finally, glucose cycling (GC) is defined as input of extracellular glucose into the glucose 6-phosphate pool followed by exit of plasma-derived glucose 6-phosphate back into the extracellular pool (22).

Calculations—The rate of glucose appearance (Rg) was calculated as the ratio of the rate of infusion of [3-H] glucose (dpm/min) and the steady-state plasma [3-H] glucose specific activity (dpm/μmol). Under steady state conditions, the rate of glucose disappearance (Rd) equals the rate of glucose appearance. The mean rate of glucose clearance (ml/kg/min) was calculated by dividing the rate of glucose disappearance (μmol/kg/min) by the ambient glucose concentration (μmol/ml). The distribution volume of water was obtained by dividing the total disintegrations/min of [3-H] O2 injected by the steady state specific activity of plasma water (dpm/ml) which was assumed to be 93% of the total plasma volume. Lean body mass was calculated from the whole body water distribution space divided by 0.73. The hepatic glucose production during the insulin clamp studies was calculated as the difference between the tracer-derived rate of appearance and the infusion rate of glucose. Rates of whole body glycolysis were estimated from the increment/unit of time in tritiated water (dpm/min × body mass (ml)/[3-H] glucose specific activity (dpm/μmol)). Regression analysis of the slopes of [3-H] H2O rates of appearance in plasma water (used in the calculation of the rate of glycolysis) was performed during the last 40-min intervals of the basal period and of the insulin clamp study, obtained from the infusion of [U-14C] lactate and [3-H] glucose. G = TGO × [3-H]UDP-glucose specific activity/[3-H]UDP-glucose specific activity/[3-H] glucose specific activity × 2. Glycogenolysis was calculated as the difference between the HGP and the gluconeogenesis. The percent of the hepatic glucose 6-phosphate pool directly derived from plasma glucose was calculated as the ratio of [3-H] UDPG and plasma [3-H] glucose specific activities. Thus, this ratio also measures the percent contribution of plasma glucose to the glucose-6-phosphatase flux (i.e. glucose cycling). Since total glucose output is equal to the sum of the HGP plus glucose cycling (and Glucose production = Glucose appearance - Glucose cycling), the term gluconeogenic rate (GC) = (HGP + Glucose appearance - Glucose cycling - total glucose output), the equation can be resolved to calculate both GC and TGO: TGO = HGP + Glucose appearance - Glucose cycling - total glucose output. It may be pointed out that this tracer methodology measures PEP-gluconeogenesis, which represents the great major part of the gluconeogenic flux under most experimental conditions. However, under these conditions the overall gluconeogenic rate under experimental conditions in which non-PEP gluconeogenesis is significantly increased.
IGF-II Transgenic Mice and Glucose Fluxes

The mean body weights between groups II and III mice (Table I). To verify the similarities between the young weight-matched negative control mice (group II) and the IGF-II transgenic mice (group III), body composition was assessed in a group of seven weight-matched 4–6-month-old negative control mice. Their average body weight was similar to groups II and III (31.9 ± 0.5 g). In these mice, lean body mass accounted for 94 ± 2% of their body weight and averaged 30.3 ± 1.0 g, while fat mass was 1.9 ± 0.7 g. The fasting plasma glucose and insulin concentrations and the basal rate of HGP were similar in the two control groups (groups I and II in Table I). However, the plasma glucose and insulin concentrations were significantly lower, and the rate of glucose appearance (HGP) and the mean rate of glucose clearance were significantly higher in the IGF-II transgenic mice compared with both control groups (Table I). The fasting plasma free fatty acid (1.49 ± 0.29 versus 1.55 ± 0.12 meq/liter) and lactate (3.8 ± 0.3 versus 3.4 ± 0.8 meq/liter) concentrations were similar in IGF-II transgenic compared with age-matched control mice. The basal plasma glucagon concentration was also unchanged in IGF-II transgenic (171 ± 23 pg/ml; range 111–223) compared with age-matched control mice (170 ± 25 pg/ml; range 109–263).

Whole Body Glucose Fluxes during the Insulin Clamp Studies—During the euglycemic clamp studies, the steady state plasma glucose and insulin concentrations were maintained at similar levels in all groups (Table II). Steady state conditions for plasma glucose concentration and specific activity and plasma insulin concentration were achieved by 40 min during the basal and clamp periods of the studies. During hyperinsulinemia, the plasma free fatty acid concentrations were similarly decreased in all groups (by ~0.9 meq/liter). Conversely, hyperinsulinemia induced a marked increase in the plasma lactate concentrations in the IGF-II transgenic mice (+0.92 ± 0.12 meq/liter), but not in the age-matched control mice (+0.08 ± 0.15 meq/liter). The rate of glucose infusion required to maintain the plasma glucose concentration at ~6.5 meq/liter was ~65% higher in the transgenic mice than in the nontransgenic controls (Table II). This marked increase in the rate of glucose disposal could be due to increased glucose uptake and/or to enhanced suppression of HGP. HGP was similarly inhibited during the euglycemic hyperinsulinemic clamp studies in all groups (65.2 ± 9.2, 70.6 ± 10.0, and 74.6 ± 12.8 μmol/kg/min in groups I, II, and III, respectively; p, not significant; Fig. 1). The rate of glucose disappearance (Rd, Fig. 2) was markedly increased in the IGF-II transgenic (533.9 ± 23.9 μmol/kg/min) compared with both control groups (340.0 ± 21.7 and 353.9 ± 27.2 μmol/kg/min, in group I and II, respectively; p < 0.01 versus III). Glycogenolysis and glycogen synthesis are the two major pathways of glucose disposal. While glycogenolysis was only marginally increased in transgenic mice, the rate of glycogen synthesis (Fig. 3) was 2-fold higher in the IGF-II transgenic (240.0 ± 26.1 μmol/kg/min) compared with both control groups (112.2 ± 22.6 and 115.0 ± 26.2 μmol/kg/min, in group I and II, respectively; p < 0.01 versus III).

The [3H]- and [14C]UDP-Glc specific activities, the [14C]PEP, and the [3H]glucose specific activities were used to calculate the contribution of plasma glucose-derived (direct pathway in Table III) and PEP-derived glucose 6-phosphate (indirect pathway in Table III) to the hepatic glucose 6-phosphate pool. Table III displays the hepatic glucose fluxes during the insulin clamp studies. While the direct pathway was similar in all groups, the portion of the hepatic glucose 6-phosphate pool formed via gluconeogenesis was ~42% higher in the IGF-II transgenic mice. Total glucose output and glucose cycling were similar in all groups (Table III). However, in transgenic mice, gluconeogenesis was significantly elevated and glycogenolysis was significantly lower than in negative control mice (Table III).

Skeletal Muscle Glycogen Concentration, Rate of [3-3H]Glucose Incorporation in Glycogen, and Glycogen Synthase Activity—The muscle glycogen concentration at the end of the insulin clamp studies was ~15% higher in the transgenic compared with the two control groups. The rate of [3-3H]glucose incorporation into skeletal muscle glycogen was ~2-fold higher in the IGF-II transgenic (6.4 ± 1.3 dpm/mg of tissue/min) than in the age- and weight-matched control groups (3.9 ± 0.7 and 3.2 ± 0.9 dpm/mg tissue/min in groups I and II, respectively). The K_m of the skeletal muscle glycogen synthase for UDPG and

---

**Table I**

General characteristics of mice receiving the euglycemic clamp studies

| Group | Glucose (mM) | Insulin (pg/ml) | GIR (pg/min) |
|-------|--------------|-----------------|--------------|
| I     | 6.4 ± 0.4    | 847 ± 28        | 275 ± 21     |
| II    | 6.8 ± 0.3    | 815 ± 34        | 283 ± 24     |
| III   | 6.5 ± 0.2    | 827 ± 39        | 495 ± 31     |

---

**Figure 1.** Rates of HGP during the basal period and during the insulin clamp studies in conscious mice. I, age-matched control (n = 9); II, weight-matched control (n = 5); III, IGF-II transgenic mice (n = 7). *, p < 0.01 versus basal; &, p < 0.01 versus I and II under the same conditions.
transgenic mice (I and II) and age (12). While IGF-II levels were almost undetectable in the livers of IGF-II homozygous mice starting at 3–4 weeks of age (12). The MUP promoter, transgene expression was demonstrated in the effects of IGF-II overproduction in adulthood from the MUP promoter (12). Use of this promoter allow one to discern human prepro-IGF-II cDNA placed under the control of the gene in adult mice. This transgenic mouse model was developed through germline insertion of a minigene containing the human prepro-IGF-II cDNA placed under the control of the MUP promoter (12). Use of this promoter allow one to discern the effects of IGF-II overproduction in adulthood from the noteworthy growth promoting effects during fetal development (5). In fact, consistent with the natural expression pattern of the MUP promoter, transgene expression was demonstrated in the livers of IGF-II homozygous mice starting at 3–4 weeks of age (12). While IGF-II levels were almost undetectable in the plasma of negative control mice, they were increased to 35–65 nm in IGF-II transgenic mice (12). Initial analyses of this model revealed the presence of hypoglycemia and lower body weight in adult homozygous mice (12). To investigate the mechanisms responsible for the alterations in glucose homeostasis in IGF-II transgenic mice, we compared conscious transgenic mice to age- and weight-matched negative control mice. The use of two control groups was necessary to account for the potential effects of altered body composition per se on the metabolic parameters. Mice were examined under basal (6 h fast) conditions and during insulin clamp studies to assess insulin sensitivity.

In the postabsorptive state, IGF-II transgenic mice were characterized by a moderate decrease in both plasma glucose and insulin concentrations, in agreement with our previous observation in 18 h fasted mice (12). Whole body glucose homeostasis is maintained by the balance between HGP and peripheral glucose utilization. Thus, postabsorptive hypoglycemia could be the consequence of either decreased HGP or increased glucose disposal. Systemic glucose clearance was increased (26) and suggests the presence of a factor stimulating glucose disposal in the absence of a significant inhibitory effect on HGP. In fact, while the increased HGP is consistent with the decrease in circulating insulin and glucose concentrations, the marked stimulation of basal glucose clearance is paradoxical. In view of the known metabolic effects of IGF on glucose metabolism in rodents (13, 17), we offer the following interpretation of the results. Circulating IGF-II levels in our homozygous transgenic mice achieved levels sufficient to promote glucose disposal in peripheral tissues (mostly skeletal muscle). The increased disposal of glucose in muscle causes a decline in the plasma glucose concentrations and an appropriate decrease in insulin secretion. The lower plasma insulin concentrations directly or indirectly bring about the increased HGP. In this regard it is likely that in the presence of basal plasma insulin concentrations skeletal muscle glycogen synthase and pyruvate dehydrogenase were partially inactive and that a sizable portion of the incoming glucose was metabolized to lactate through anaerobic glycolysis. The absence of a significant increase in the circulating lactate concentrations may be explained by concomitant increases in both peripheral lactate release and lactate utilization via gluconeogenesis.}

![Fig. 2. Rates of glucose disappearance during the basal period and during the insulin clamp studies in conscious mice. I, age-matched control (n = 9); II, weight-matched control (n = 5); III, IGF-II transgenic mice (n = 7). *p < 0.01 versus basal; &p < 0.01 versus I and II under the same conditions.](image)

![Fig. 3. Pathways of intracellular glucose disposal during the insulin clamp studies in conscious mice. Rates of glycolysis were derived from the rate of conversion of [3–H]glucose to H2O (12). Rates of glycogen synthesis were estimated by subtracting the rate of glycolysis from the Rg, I, age-matched control (n = 9); II, weight-matched control (n = 5); III, IGF-II transgenic mice (n = 7). &p < 0.01 versus I and II under the same conditions.](image)

**DISCUSSION**

The purpose of the recent study was to delineate the metabolic consequence of the overexpression of the human IGF-II gene in adult mice. This transgenic mouse model was developed through germline insertion of a minigene containing the human prepro-IGF-II cDNA placed under the control of the MUP promoter (12). Use of this promoter allow one to discern the effects of IGF-II overproduction in adulthood from the noteworthy growth promoting effects during fetal development (5). In fact, consistent with the natural expression pattern of the MUP promoter, transgene expression was demonstrated in the livers of IGF-II homozygous mice starting at 3–4 weeks of age (12). While IGF-II levels were almost undetectable in the

---

**TABLE III**

| Group | I | II | III |
|-------|---|----|-----|
| Direct pathway (%) | 23.6 ± 3.2 | 25.7 ± 1.1 | 24.6 ± 3.1 |
| Indirect pathway (%) | 33.2 ± 6.2 | 32.6 ± 5.4 | 46.7 ± 6.1 |
| TGO (µmol/kg·min) | 91.9 ± 9.2 | 94.4 ± 16.7 | 98.9 ± 13.9 |
| Glucose cycling (µmol/kg·min) | 26.7 ± 3.2 | 23.8 ± 4.7 | 24.3 ± 3.9 |
| Gluconeogenesis (µmol/kg·min) | 26.4 ± 4.2 | 29.4 ± 5.7 | 46.2 ± 3.9 |
| Glycogenolysis (µmol/kg·min) | 38.8 ± 3.2 | 41.2 ± 5.0 | 28.4 ± 3.9 |

* Direct, percent of the hepatic G6P pool derived from plasma glucose, calculated as the ratio of the specific activities of [3–H]UDP-Glu (Glu) or [3–H]UDP-Gal (Gal) and [3–H]Glu.

* Indirect, percent of the hepatic G6P pool derived from PEP-gluconeogenesis, calculated as the ratio of the specific activities of [3–H]UDP-Glu (Glu) or [3–H]UDP-Gal (Gal) and [3–H]PEP.

* p < 0.01 versus groups I and II.

* TGO, total glucose output (flux through glucose-6-phosphatase).
nally, although hypoglycemia may also trigger hormonal counter-regulation with increases in plasma glucagon and free fatty acid concentrations, we were unable to demonstrate such an effect in IGF-II transgenic mice. Thus, the postabsorptive hypoglycemia was due to increased systemic clearance of glucose. Overall, this observation in IGF-II transgenic mice closely resembles the physiologic profile of transgenic mice overexpressing the human GLUT4 or GLUT1 protein in insulin target tissues (27–30).

To directly examine the sensitivity of tissue glucose uptake and HGP to insulin in IGF-II transgenic mice, we performed euglycemic hyperinsulinemic (~850 microunits/ml) clamp studies in conscious mice. Whole body glucose utilization (Rg) and the rate of glucose infusion required to maintain euglycemia were 55–65% higher in IGF-II transgenic mice compared with both negative control groups in the presence of equal plasma glucose and insulin concentrations. This marked increase in insulin-stimulated glucose uptake (~180–194 μmol/kg min) cannot be solely explained by the increased rate of basal turnover (~38–59 μmol/kg min). We also examined the two major pathways of intracellular glucose disposal. While both glycolysis and glycogen synthesis were significantly increased in IGF-II transgenic mice, the increased glycogen deposition accounted for ~70% of the increased Rg. This improvement in the efficiency of glycogen synthesis during hyperinsulinemia was confirmed at the tissue level. In fact, the rate of [3-3H]glucose incorporation in skeletal muscle glycogen was ~2-fold increased in the IGF-II transgenic compared with both negative control groups. Thus, in the presence of hyperinsulinemia, the excess of glucose taken up in skeletal muscle is efficiently stored in muscle glycogen. The increased rates of glycogen synthesis during periods of relative hyperinsulinemia (postprandially) in IGF-II transgenic mice may provide substrates for the enhanced gluconeogenesis and HGP during the following postabsorptive fasting periods. While basal HGP was significantly increased in the transgenic mice, its inhibition during the hyperinsulinemic clamp studies was similar to control. The paucity of type I IGF receptors in the adult liver (31) may account for the inability of the elevated IGF-II concentrations to restrain the hepatic production of glucose. The similar HGP in the presence of equal plasma insulin and glucose concentrations provides further support for the notion that the increased HGP under basal conditions is secondary to the lower plasma glucose and insulin concentrations. However, it is of interest that while the negative control mice derived 60% of the HGP from glycogen breakdown, the IGF-II transgenic mice were characterized by a marked increase in gluconeogenesis, which comprised >60% of HGP. While we failed to demonstrate significant alterations in basal plasma glucagon concentrations or in the activity of key hepatic enzymes, such as glucokinase, glucose-6-phosphatase, and phosphoenolpyruvate carboxykinase, there was a marked increase in the plasma lactate concentrations during the insulin clamp studies in the IGF-II transgenic mice but not in age-matched control mice. Importantly, this occurred despite a likely increase in hepatic lactate utilization for gluconeogenesis. Indeed, an increase in the availability of gluconeogenic precursors has been shown to result in enhanced gluconeogenesis in the absence of changes in HGP (32). Thus, the hepatic overexpression of IGF-II in postpuberal mice leading to a 20–30-fold increase in circulating IGF-II concentrations increased basal and insulin-stimulated glucose disposal, muscle glycogen synthesis, and hepatic gluconeogenesis.

Although it is likely that these metabolic changes are due to high circulating IGF-II levels per se, some alternative hypothesis should also be considered. The sustained increase in plasma IGF-II concentrations is expected to lower growth hormone (GH) secretion. A decrease in GH may contribute to some of the observed changes in body composition and insulin sensitivity. Although we did not directly measure GH levels in these mice, the modest and inconsistent alterations in IGF-I and IGF-binding proteins concentrations previously reported in these mice (12) appear to indicate that the GH levels were maintained to near-normal levels. Furthermore, the administration of GH to aging humans has been associated with increased lean body mass and decreased fat mass, suggesting a role of GH deficiency for the relative increase in fat mass in human aging (10). These effects of the hormone are opposite to those observed in this transgenic model. It is also possible that the marked metabolic impact of the postpuberal increase in IGF-II gene expression is mediated by its effects on body composition. In fact, the body weight and composition of 8–10-month-old IGF-II transgenic mice (12) closely resembles those of younger (4–6-month-old) negative controls. In fact, lean body mass comprises a larger portion (91–97% versus 84%) of body weight in IGF-II transgenic mice compared with age-matched negative control mice. However, the observation that the rates of basal and insulin-stimulated glucose disposal were markedly increased in IGF-II transgenic mice compared with young weight-matched negative controls suggest that other factors contribute to the metabolic changes in this transgenic model. Conversely, it may be speculated that the changes in body composition are the consequence of the sustained metabolic actions of IGF-II in this transgenic model. In fact, since IGF-II displays higher affinity for the IGF-I than for the insulin receptors (33), it may exert more potent effects on protein anabolism than on lipogenesis compared with insulin (17). The concomitant decrease in circulating insulin levels is likely to contribute to decreased net lipogenesis. Recent data from transgenic mice with overexpression of the glucose transporter subtype, GLUT4, in adipose cells suggests that in vivo glucose flux may regulate fat accretion in mice (30). Thus, a relative increase in the disposal of glucose in skeletal muscle versus adipose tissue may also contribute to decreased fat mass and preserved lean body mass in IGF-II transgenic mice. Finally, the independent hypoglycemic effects of IGF-II are supported by the observation of tumor-associated hypoglycemia in patients with increased IGF-II gene expression in tumor cells (34). Although there is no conclusive evidence for an age-related decrease in plasma IGF-II concentrations in humans, the reported decline in IGF-I levels (10, 11) is likely to determine an overall decrease in IGF action in human aging (8–11).

In conclusion, the postpuberal increase in IGF-II gene expression in mouse liver generating plasma IGF-II levels comparable with those of adult humans causes a marked improvement in glucose homeostasis with increased basal and insulin-stimulated rates of glucose disposal in adult transgenic mice. It is suggested that IGF-II has a physiologic metabolic function in adult humans.

Acknowledgments—We thank Gary Sebel and Rong Liu for excellent technical assistance.

REFERENCES

1. Rinderknecht, E., and Humbel, R. E. (1978) J. Biol. Chem. 253, 2769–2776
2. Frosch, E. R., and Zapf, J. (1981) Diabetesologia 28, 485–493
3. Daughaday, W., and Rotwein, P. (1989) Endocrinol. Rev. 10, 68–91
4. Zapf, J., Schoenlie, E., and Frosch, E. R. (1978) Eur. J. Biochem. 87, 285–296
5. Stylianopoulou, F., Efstratiadis, A., Herbert, J., and Pinta, J. (1988) Develop. 103, 497–506
6. DeChiara, T., Efstratiadis, A., and Robertson, E. J. (1990) Nature 345, 78–80
7. Adams, S. O., Nisley, S. P., Handwearger, S., and Reher, M. M. (1993) Nature 302, 150–153
8. Cohen, P., Orant, I., Fielder, P. J., Neely, E. K., Gargosky, S. E., Dea, C. I., Cada, P., Youngman, O., Pharr, H., Lanson, G., Giudice, L. C., and Rosenfeld, R. G. (1992) Psychoneuroendocrinology 17, 335–342
9. Bennett, A. E., Wahner, H. W., Riggs, B. L., and Hinz, R. L. (1984) J. Clin.
IGF-II Transgenic Mice and Glucose Fluxes

1. Rudman, D., Feller, A. G., Nagraj, H. S., Gergans, G. A., Lalitha, P. Y., Goldberg, A. F., Schlenker, R. A., Cohn, L., Rudman, I. W., and Mattson, D. E. (1990) N. Engl. J. Med. 323, 1–6
2. Kupfer, S. R., Underwood, L. E., Baxter, R. C., and Clemmons, D. R. (1993) J. Clin. Invest. 91, 391–396
3. Rogler, C. E., Yang, D., Rossetti, L., Donohoe, J., Alt, E., Donohoe, J., Chang, C. J., Rosenfeld, R., Neely, K., and Hintz, R. (1994) J. Biol. Chem. 269, 13779–13784
4. Stumpel, F., and Hartmann, H. (1992) Diabetologia 35, 932–938
5. Burguera, B., Elton, C. W., Tapscott, E. B., Pories, W. J., DiMarchi, R., Sakano, K., and Dohm, G. L. (1994) Biochem. J. 300, 781–785
6. Czech, M. P. (1989) Cell 59, 235–238
7. Jacob, R., Barrett, E., Plewe, G., Fagin, K. D., and Sherwin, R. S. (1989) J. Clin. Invest. 83, 1717–1726
8. Rossetti, L., Smith, D., Shulman, G. I., Papachristou, D., and DeFronzo, R. A. (1987) J. Clin. Invest. 79, 1510–1515
9. Rossetti, L., and Laughlin, M. R. (1989) J. Clin. Invest. 84, 892–899
10. Rossetti, L., Frontoni, S., DiMarchi, R., DeFronzo, R. A., and Giaccari, A. (1993) Diabetes 40, 444–448
11. Giaccari, A., and Rossetti, L. (1992) J. Clin. Invest. 89, 36–45
12. Rossetti, L., Giaccari, A., Barzilai, N., Howard, K., Seibel, G., and Hu, M. (1993) J. Clin. Invest. 92, 1126–1134
13. Barzilai, N., and Rossetti, L. (1993) J. Biol. Chem. 268, 25019–25025
14. Rossetti, L., and Giaccari, A. (1990) J. Clin. Invest. 85, 1785–1792
15. Giaccari, A., and Rossetti, L. (1989) J. Chromatogr. 497, 69–78
16. Cryer, P. E. (1993) Am. J. Physiol. 264, E149–E155
17. Gulve, E. A., Ren, J. M., Marshall, B. A., Gao, J., Hansen, P. A., Holloszy, J. O., and Mueckler, M. (1994) J. Biol. Chem. 269, 18366–18370
18. Ren, J. M., Marshall, B. A., Mueckler, M. M., McCaleb, M., Amatruda, J. M., and Shulman, G. I. (1995) J. Clin. Invest. 95, 429–432
19. Treadway, J. L., Hargrove, D. M., Nardone, N. A., McPherson, R. K., Russo, J. F., Milic, A. J., Stukenbrok, H. A., Gibbs, E. M., Stevenson, R. W., and Pessin, J. E. (1994) J. Biol. Chem. 269, 29956–29961
20. Shepherd, P. R., Gnudi, L., Tazzo, E., Yang, H., Leach, F., and Kahn, B. B. (1993) J. Biol. Chem. 268, 22243–22246
21. Caro, J. F., Poulos, J., Ittooq, O., Pories, W. J., Flickinger, E. G., and Sinha, M. K. (1988) J. Clin. Invest. 83, 976–981
22. Jennisen, T., Nurjhan, N., Consoli, A., and Gerich, J. E. (1990) J. Clin. Invest. 86, 489–497
23. Lefrath, D., Sampson, P. C., and Roberts, C. T. (1994) HormoneRes. 41, Suppl. 2, 74–78
24. Gorden, P., Hendricks, C. M., Kahn, R. C., Megyesi, K., and Roth, J. (1981) N. Engl. J. Med. 305, 1452–1455