The physiological role of insulin-like growth factor (IGF) II (IGF-II) in adult humans is poorly understood. Rather high levels of IGF-II persist in adult human serum, whereas, in rodents, IGF-II levels are very low. To investigate the physiological and carcinogenic effects of persistently elevated IGF-II in adults, we have produced two lines of transgenic mice in which high levels of IGF-II (20- or 30-fold increase above normal) are persistently maintained in the blood. The transgene is driven by the major urinary protein promoter, and it is highly expressed in the liver and perputial glands in both lines. The adult transgenic mice are smaller than controls, and their body composition is altered. Their lean body mass is reduced by 5-8%, whereas fat mass is reduced between 44 and 77%. The mice expressing the highest level of IGF-II (60%) develop hypoglycemia and hypoinsulinemia and IGF-I levels are normal. Mice in the lower expression line (20-fold elevated IGF-II) develop hypoglycemia progressively over their lifetime. Mice of both lines also develop a diverse spectrum of tumors at a higher frequency than controls after 18 months of age, and the most frequent types of tumors are hepatocellular carcinomas and lymphomas. Squamous cell carcinoma, sarcoma, and thyroid carcinomas also occurred in our test group. The long latent period before tumors arise and the wide spectrum of tumor types suggest that IGF-II may function primarily as a tumor progression factor in mice via autocrine and endocrine mechanisms of action.

Altered Body Composition and Increased Frequency of Diverse Malignancies in Insulin-like Growth Factor-II Transgenic Mice*

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Insulin-like growth factor (IGF) II (IGF-II) is a member of the insulin family which also includes insulin, IGF-I, and relaxin. The IGFs exert insulin-like effects on glucose and fat metabolism and promote the growth of many cell types in culture (1-6). Treatment of adult rats with recombinant IGF-II induced hypoglycemia, along with enhanced tissue uptake of 2-deoxyglucose, enhanced glycogen formation in skeletal muscle, and lipogenesis in epididymal fat (7). Serum concentrations of recombinant IGF-II which induced insulin-like responses ranged from 40 to 70 nmol (7) which was approximately 50 times the level of insulin needed to induce the same effects. At least half of the recombinant IGF-II in the blood remained in the “free” form (7). IGF-II binds with high affinity to a unique, bifunctional receptor which also functions as the mannose 6-phosphate receptor (referred to as the IGF-II/Mann-6-P receptor) (8). In addition, it cross-reacts with both insulin and IGF-I receptors (9). Since the serum IGF-II concentrations needed to mimic insulin action exceed the reported affinity of IGF-II for the IGF-II/Mann-6-P receptor severalfold (10), the acute metabolic actions of IGF-II may be mediated through its cross-reaction with insulin receptors or IGF-I receptors (11). In vivo studies with rat hepatocytes, which lack IGF-I receptors (12), support an insulin receptor-mediated action of IGF-II on glucose metabolism (13).

The IGF-II gene is expressed in many tissues during embryonic and newborn development (14), whereas expression in adult rodents (mice, rats) is confined to the choroid plexus and the leptomeninges (15). Gene targeting experiments have clearly established IGF-II as an important embryonic growth factor in mice, since mice which are homozygous null for IGF-II have low birth weights (16). Conversely, deletions in chromosome 7 of the mouse which lead to a complete loss of the IGF-II/Mann-6-P receptor cause an increase in fetal birth weight (17). It has, therefore, been postulated that the main function of IGF-II/Mann-6-P receptors located on the cytoplasmic membrane, as far as IGF-II is concerned, may be to clear IGF-II from the serum and target it for degradation in lysosomes (18, 19). Therefore, binding of IGF-II to IGF-I and/or insulin receptors is also thought to be the primary mechanism by which it exerts its growth-promoting effects (11).

The importance of regulating IGF-II biological activity is underscored by the existence of at least four negative regulatory mechanisms to control IGF-II biological activity during fetal and adult life. These include 1) transcriptional repression by the Wilms’ tumor suppressor gene (WT1) (20), which is located on chromosome 11p13; 2) imprinting, which inactivates the maternal IGF-II allele (21); 3) clearance from the circulation by IGF-II/Mann-6-P receptors (17); and 4) the presence of at least six IGF-binding proteins in blood (22). Depending on the specific cell type and IGF-binding protein, the IGFBPs may either enhance IGF-II effects on metabolism and growth or interfere with IGF-II activity by preventing interaction with specific cell receptors (23-26). The complex array of interactions between IGFs and the IGFBPs in vivo are just beginning to be understood.

In spite of several negative controls, IGF-II is highly overexpressed in many diverse types of tumors (27-29), and large tumors which secrete IGF-II can induce hypoglycemia (29-31). IGF-II is also overexpressed in the earliest precancorous le-
sions identified in an animal model for hepatocellular carcinoma, suggesting that IGF-II expression may be an early event in those tumors (32). Studies using cultured Balb/c 3T3 cells have shown that IGF-II is more active in promoting cell growth when it functions together with other growth factors, such as epidermal growth factor, which have rendered cells competent (33, 34). IGF-II expression in MCF-7 cells promotes malignant progression of the cells (35). Although these studies suggest a role for IGF-II in a carcinogenesis, the question whether it plays a causal role in malignant transformation in vivo has remained.

To circumvent the normal negative controls on IGF-II expression in adult mice and directly assess its metabolic, growth-regulating, and carcinogenic activities, we have produced transgenic mice in which the human prepro-IGF-II cDNA (36) was placed under the control of the major urinary protein (MUP) promoter (37). This promoter is normally silent until puberty in male mice, at which time male hormones induce its expression to high levels, primarily in the liver (37, 38). Use of this promoter avoids possible lethal effects of IGF-II overexpression in the fetus. Results presented in this report demonstrate that persistent elevation of IGF-II causes a lowering in body weight which is predominantly due to a reduction in body fat and that IGF-II causes an increase in the frequency of diverse types of tumors which arise late in life in the mice.

**EXPERIMENTAL PROCEDURES**

**Construction of the Transgene**—The transgene was constructed by first inserting the SV40 sequences into the EcoRI site of the MUP vector reported by Held et al. (37). This new MUP-SV40 vector was then digested with XbaI and blunt-ended with T4 polynucleotide, and a blunt-ended propro-IGF-II cDNA (Paf1 fragment) (36) was inserted into the blunt-ended XbaI site in the sense orientation. A Paf1-EcoRI transgene fragment was purified and used for production of transgenic mice (see Fig. 1).

**Northern Blot Analysis**—Fifteen micrograms of liver RNA were loaded per lane, and transgene expression was detected on a Northern blot using a "3P-labeled IGF-II cDNA probe generated by random priming of a human prepro-IGF-II cDNA insert (27).

**Western Blot Analysis**—Acid ethanol extracts of liver were prepared as described previously (32), and polypeptides were separated by polyacrylamide gel electrophoresis through a 15% polyacrylamide gel, followed by immunological detection, using a rabbit anti-human IGF-II antisera (Fig. 1C, lanes 1-5) or normal rabbit antisera (Fig. 1C, lanes 6 and 7) and autoradiographic detection after incubation with 125I-labeled protein A as previously reported (32). The woodchuck protein sample (Fig. 1C, lane 5) was from an IGF-II-positive liver tumor to which 150 ng of recombinant 7.5-kDa human IGF-II were added as a positive control and molecular mass marker. The IGF-II antisera was a gift from Lilly.

**Analysis of Growth Curves**—Body weight was determined at 2-week intervals for approximately 9 months for 117 mice (line 23), 96 mice (line 25) and 123 mice (negative control). Statistical analysis was carried out by stratifying the data into age categories (0.5-4, 5-8, 9-12, and 13-16 months) in order to fit either quadratic or linear polynomial functions to the varying slopes of the curves. The quadratic equations which were fit to the 0.5-4- and 5-8-month portions of the growth curves were Equations 1 and 2, respectively (see Table I). The linear equations fit to the 9-12- and 13-16-month portions of the curves were Equations 3 and 4, respectively (see Table I). Line 25 mice were smaller than controls in all age categories with p values of 0.001, <0.0001, and <0.0001, respectively. MUP line 23 mice were significantly smaller than controls only in the 5-8-month age category (p = 0.0368), and in the age category 9-12 months the p value was 0.1105 (nearly significant). In the other age categories the p values were much higher, revealing no significant difference in body weight. Statistical analysis was conducted using a program designed to take into account repeated measurements of individual animals of different ages (39, 40). We used the generalized estimating equations (GEE) approach to repeated measurements derived by Liang and Zeger (40). This method applied the generalized linear model theory to the data set with irregular numbers of repeated measurement observations. By specifying the "working" correlation matrix among the individual operations, this GEE method gives the univariate regression type analysis. For the data set we used either linear or quadratic polynomial functions to fit the growth curve, depending on which was the best fit of the data, and the p values were generated by comparing line 23 or line 25 data with the negative control data. The IGF-II, insulin, glucose, and lean body mass measurements were compared using the unpaired t test, and the averages are listed ± 1 S.E. of the mean. p values of 0.05 or below were judged to be significantly different from controls.

**Blood samples for IGF, insulin, and glucose measurements were obtained by bleeding from the retroorbital sinus after an overnight fast. Plasma samples were prepared and stored frozen. Equal amounts of plasma from several mice (n = 2-7) were pooled if necessary to obtain adequate sample volume. Plasma samples were analyzed as described below for IGFs, insulin, and glucose determinations. For IGF determinations, 100-μl samples were chromatographed in 0.25 M formic acid on a Sephadex G-50 column (Pharmacia Biotech Inc.). Peptide fractions were collected in 1% bovine serum albumin, frozen, and lyophilized. Radioimmunoassays (RIAs) were used to determine IGF levels as previously reported (41, 42). Briefly, RIAs for IGF-I utilized 125I-IGF-I as radioligand and polyclonal antiserum U8K 467; IGF-II RIAs utilized 125I-IGF-II and a monoclonal anti-rat IGF-II antibody (Amano Enzyme, Troy, VA). The column procedure separated IGF-binding proteins and allowed accurate determination of IGF levels by RIA as described above. The IGF value of each plasma sample was the average of triplicate RIA measurements. In the case of line 23 mice, eight plasma samples representing plasma pooled from 30 mice were analyzed. In the case of line 25 mice, 10 plasma samples representing pooled plasma from 44 mice were assayed, and 12 plasma samples pooled from 58 negative mice were assayed to determine negative control levels. IGF levels listed in Table II represent the weighted average of the experimental determinations. IGF levels were nearly constant over the life of the mice (data not shown), and IGF values reported here represent the average from mice between 2 and 20 months of age.

**Inulin levels were determined in fasted plasma samples of mice between 4 and 5 months of age by RIA using kits obtained from Linco. n = 11 for each group.**

**Glucose levels in plasma samples were determined using a Beckman glucose analyzer.**

**Body composition was determined by the method previously reported (43). Basically the method utilized the steady state distribution of H2O in blood after a bolus injection of 1 μCi of H2O per animal. Lean body mass was inversely proportional to the amount of H2O retained in the blood between 2 and 3 h after injection. Six- to nine-month-old male mice were utilized for the tests (n = 19-26 per group).**

### Table I

| Analysis of growth curves |
|---------------------------|
| Quadratic equations 1      |
| E(weight) = 1.61           |
| group L23                  |
| E(weight) = 0.69           |
| group L25 + 15.84 (age) = 2.27 (age)² |
| group negative             |
| Quadratic equations 2      |
| E(weight) = 21.49          |
| group L23                  |
| E(weight) = 19.52          |
| group L25 + 2.95 (age) = 0.166 (age)² |
| group negative             |
| Linear equations 3         |
| E(weight) = 31.96          |
| group L23                  |
| E(weight) = 27.85          |
| group L25 + 0.61 (age)     |
| group negative             |
| Linear equations 4         |
| E(weight) = 41.50          |
| group L23                  |
| E(weight) = 35.85          |
| group L25 - 0.13 (age)     |
| group negative             |
IGF-II Transgenic Mice

RESULTS

To develop an animal model to study the metabolic, growth regulatory, and carcinogenic effects of IGF-II, we have produced transgenic mice in which the human prepro-IGF-II cDNA was placed under the control of the MUP promoter (37). The SV40 small T intron and poly(A) addition signal were added to the 3' end of the transgene to promote translation (Fig. 1A). Two lines of transgenic mice were obtained and were designated MUP-IGF-II line 23 and MUP-IGF-II line 25 (referred to in this report as line 23 or line 25). Both lines express high levels of the transgene in the liver and peripartial glands. Low expression also occurs in the adrenal gland of each line. Western blotting of acid-ethanol extracts of liver detected a protein of approximately 14-16 kDa (Fig. 1C), which is the size expected for partially processed pro-IGF-II (44, 45). Liver protein extracts from heterozygous mice contained less IGF-I1 than extracts from homozygous mice (Fig. 1C, lanes 1 versus 2, respectively). The IGF-II polypeptides present in the livers of the transgenic mice was the same size as IGF-II polypeptides present in a hepatocellular carcinoma from a woodchuck hepatitis virus (WHV) carrier (Fig. 1C, lanes 1-4 versus lane 5). Recombinant human IGF-II was added to the woodchuck liver protein extract as a positive control and appears as a 7.5-kDa band (marked with an asterisk) in Fig. 1C, lane 5.

IGF-II levels in the blood of 2-20-month-old homozygous line 23 male mice averaged 454 ng/ml (60.4 nm) (Table II), whereas IGF-II in the blood of heterozygous line 23 males averaged 243 ng/ml (32.4 nm). Negative control mice averaged only 15 ng/ml (2 nm) IGF-II in their blood at similar ages. The average level of serum IGF-I1 in homozygous male line 25 mice was 316 ng/ml (42 nm), which was significantly lower than the level in line 23 mice (p = 0.0001), consistent with a lower transgene copy number in that line (Table II). Size fractionation of serum IGF-I1 by acid P60 columns (30), showed that the IGF-II in blood was predominantly 7.5 kDa, consistent with it being the fully processed mature polypeptide (data not shown). These serum IGF-II levels were in the same range as those required for exogenous recombinant IGF-II to exert its half maximal metabolic effects in adult rats (7).

The steady state fasting insulin and blood glucose levels in male line 23 mice are approximately 20% lower than nontransgenic controls, and the mice are therefore moderately hypoglycemic and hypoinsulinemic. In contrast, steady state glucose and fasting insulin levels were normal in young line 25 mice (age 10 months), whereas IGF-I levels were reduced approximately 20% (Table II). Subsequent analysis of the steady state fasting glucose levels in older line 23 and 25 mice (up to 19 months) has shown that line 23 mice maintain the same level of hypoglycemia (80 mg/dl glucose versus 101 mg/dl in controls) over their lifetime, whereas line 25 mice develop hypoglycemia progressively as they get older. At 19 months line 25 mice have the same degree of hypoglycemia (~50 mg/dl) as line 23 male mice.

In some experimental systems IGFBPs interfere with the biological activity of IGFs, and in other experimental systems they enhance IGF actions (23-25). Since some in vitro studies also have demonstrated IGF regulation of IGFBP gene expression, we assessed the relative levels of IGFBPs in plasma of homozygous and heterozygous male transgenic mice using li-

FIG. 1. A, structure of the MUP-IGF-II transgene. Nucleotide numbers above the human prepro-IGF-II sequences are those from Bell et al. (36), the nucleotide numbers above the SV40 DNA sequences correspond to those in the established SV40 map, and the Major Urinary Protein (MUP) promoter used was that reported earlier by Held et al. (37). The Pre, BCAD, and E domains of IGF-II are noted within the coding sequence of the human IGF-II cDNA. B, transgene expression in the liver of male and female homozygous line 23 and line 25 transgenic mice between 1 and 4 weeks old. Odd-numbered lanes, males; even-numbered lanes, females. Lanes 1-5, line 25 mice; lanes 9-12, line 23 mice. Age of mice, lanes 1 and 2, 1 week; lanes 3 and 4, 2 weeks; lanes 5, 6, 9, and 10, 3 weeks; lanes 7 and 8, 11, and 12, 4 weeks. Thin arrow at left denotes endogenous IGF-II mRNA present in liver of 1- and 2-week-old mice. Thick arrow, at right, denotes the transgene mRNA. Note the absence of transgene mRNA at 2 weeks, in line 25 and at 3 weeks in line 23, and its high level in the following weeks. C, detection of IGF-II polypeptides in transgenic mouse livers. Lanes 1 and 2, liver protein samples from line 23 male heterozygous or homozygous mice, respectively. Lanes 3 and 4, liver protein samples from line 25 male heterozygous or homozygous mice, respectively. In lane 4, less protein was loaded. Lane 5, liver protein sample from a HCC from a WHV-carrier woodchuck. Recombinant human IGF-II, labeled with an asterisk in lane 5, was added to the woodchuck liver protein extract as a positive control. Lanes 6 and 7, acid ethanol extract of liver protein from line 23 (lane 6) and line 25 (lane 7) mice reacted with normal rabbit serum.
The case of line 23, analysis of the growth curve revealed a control mice was statistically significant in each case. We estimated the body fat and lean body mass. The average distribution of tritiated water to measure lean body mass (43), dramatic reduction in epididymal fat (data not shown). Brown (Table gous male mice ate significantly more food than control mice and in all the age categories in the respective age periods was 5, 11, 15, and 17% (Fig. 2). In <0.0001 and 0.0011), and the average reduction in body weight lean body mass was 30 g for line 23 and 30.8 g for line 25 mice, old homozygous male mice since both lines 23 and 25 were body mass of transgenic and control mice. We chose 6-9-month-old lines of our IGF-I transgenic mice for several years. We rarely compared to 32.5 g for controls. This slight, but significant, loss mass. Average whole body fat mass for line 23 mice was 3.5 g and blots (46, 47). In heterozygous males, we did not observe any significant alterations in the IGFBPs. However, in homozygous males of both line 23 and 25, we observed a 70 and 40% increase, respectively, in IGFBPs which migrate at the position of IGFBPs 1, 2, and 5 in the blots. No change in IGFBPs which migrate in the positions of IGFBP-3 or IGFBP-4 was detected (data not shown).

We originally hypothesized that a major effect of chronic IGF-II over-production by the liver might be to promote growth of adolescent and adult mice. We conducted a long term growth study with homozygous male mice of lines 23 and 25 and compared the growth curves to negative control mice. The growth of approximately 100 mice in each group was followed, and the statistical analysis of body weight data was carried out by stratifying the data into different age categories (0.5-4, 5-8, 9-12, and 13-16 months) in order to fit either quadratic or linear polynomial functions to the growth curve data using the GEE approach (39, 40). Line 25 mice were significantly smaller than controls in all the age categories (p values between <0.0001 and 0.0011), and the average reduction in body weight in the respective age periods was 5, 11, 15, and 17% (Fig. 2). In the case of line 23, analysis of the growth curve revealed a statistically significant 6% reduction of body weight between 5 and 8 months (p = 0.0368) and a 5% reduction between 9-12 months (p = 0.1105).

To examine the components of body mass which are most affected by chronic IGF-II elevation, we determined the lean body mass of transgenic and control mice. We chose 6-9-month-old homozygous male mice since both lines 23 and 25 were significantly smaller during that period. Using the steady state distribution of tritiated water to measure lean body mass (43), we estimated the body fat and lean body mass. The average lean body mass was 30 g for line 23 and 30.8 g for line 25 mice, compared to 32.5 g for controls. This slight, but significant, loss of lean body mass was contrasted by a dramatic reduction in fat mass. Average whole body fat mass for line 23 mice was 3.5 g and line 25 was only 1.5 g compared to 6.6 g for control mice (Table II). The difference in fat mass between transgenic and control mice was statistically significant in each case (p values of 0.0128 and 0.0001 for lines 23 and 25, respectively). Further analysis of specific fat deposits in the mice have revealed a dramatic reduction in epididymal fat (data not shown). Brown fat has not yet been measured.

Hypoglycemia might cause animals to eat more so we conducted a daily food intake study. As expected, line 23 homozygous male mice ate significantly more food than control mice (Table II). Interestingly, line 25 homozygous male mice ate significantly less than controls, and they also had the greatest reduction in body weight and fat mass. Hypotheses to explain these results are presented under "Discussion."

We have followed the occurrence of malignancies in both lines of our IGF-II transgenic mice for several years. We rarely observe any tumors in the mice under the age of 18 months. However, when mice 18 months and older (to approximately 24 months old) were sacrificed we observed a diverse spectrum of tumors at a significantly higher frequency (22/101) in the IGF-II transgenic mice than in controls (4/59) according to the Pearson chi square test (Table III; p = 0.013). When the data were stratified according to whether the mice were homozygous or heterozygous for the transgene, homozygous mice had the highest frequency of tumors and this was significantly different from control (p = 0.005). Among the homozygous animals, the frequency of tumors in males was significantly higher than in females (p = 0.025). Thus, the animals with the highest dosage of the transgene developed tumors at the highest frequency, and females which had a lower expression of the transgene (especially later in life) also had a lower incidence of tumors. So far, we have observed a wide variety of tumors which arise in the mice. The most frequent tumors are hepatocellular carcinomas and lymphomas (Table III). Other types of tumors which have occurred so far include squamous cell carcinoma, sarcomas, thyroid carcinoma, and several tumors whose origin has not yet been determined. Sarcomas and HCCs, which express IGF-II, have previously been linked to non-islet cell tumor-induced hypoglycemia (29-31). Tumors arise in organs of the mice which express the transgene (liver) as well as organs

### Table II

| Negative control | Line 23 | Line 25 |
|------------------|---------|---------|
| IGF-II (ng/ml)   | 15 ± 0.91 | 454 ± 12.16* | 316 ± 13.12* |
| IGF-I (ng/ml)    | 185 ± 11.55 | 177 ± 10.79* | 144 ± 8.59 |
| Insulin (ng/ml)  | 0.44 ± 0.04 | 0.33 ± 0.02 | 0.40 ± 0.04 |
| Glucose (mg/dl)  | 101 ± 2.7 | 78 ± 3.2 | 104 ± 3.9* |
| Body weight (g)  | 38.7 ± 0.9 | 33.0 ± 0.6 | 31.8 ± 0.6 |
| Lean body mass (g) | 32.5 ± 0 | 30.0 ± 0.6 | 30.8 ± 0.4 |
| Fat mass (g)     | 6.6 ± 0.8 | 3.5 ± 0.8 | 1.5 ± 0.3 |
| Food intake, g/day | 5.1 ± 0.3 | 6.4 ± 0.3 | 4.2 ± 0.2 |

* Superior numbers 1-9 refer to methods given in numbered paragraphs under "Experimental Procedures."

† Applies only for 1-10-month-old mice, older mice progressively develop hypoglycemia which is equivalent to line 23 mice.

**Fig. 2. Curves depicting body weight versus age for homozygous male line 23 and line 25 mice versus negative controls.**
Tumor frequency: numerator equals the number of animals with a tumor and denominator is the total number of animals sacrificed in that category. Frequencies were compared to negative controls in the respective categories as listed. $p$ values of 0.05 or below are judged to be significantly different from control. Types of tumors: tumor types were determined by histological analysis of hematoxylin and eosin stained slides from formalin fixed, paraffin embedded tissues. The frequency of each tumor type in each category is given in parentheses. In cases in which the tumor type could not be readily determined, yet the tissue was definitely malignant, the tumor is listed as "unknown type."

**DISCUSSION**

Line 23 homozygous male mice have a 30-fold increase in IGF-II in the blood, whereas homozygous male line 25 mice have a 20-fold increase. The IGF-II levels in line 25 mice are in the minimum range of those needed for inducing insulin-like effects in rats using recombinant IGF-II (42 nm), whereas the levels in line 23 mice (60 nm) are near those which induce maximal insulin-like effects (7). Therefore, it might have been predicted that homozygous male line 23 mice would develop chronic hypoglycemia and hypoinsulinemia, as is the case. In contrast, the level of IGF-II in line 25 mice is not sufficient to induce hypoglycemia in young animals. Instead, hypoglycemia develops gradually during the lifetime of line 25 mice. Line 25 homozygous male mice also eat less than control mice (when given food ad libitum) and have a greater reduction in body weight than line 23 mice. Line 23 males eat more than controls as would be expected due to their chronic hypoglycemic state, and this may account for the overall smaller reduction in body weight and fat mass compared to line 25 mice.

The most striking common metabolic effect of IGF-II in our lines is the large reduction in body fat. While in control mice fat mass averaged 17% of their body weight, in homozygous male line 23 and line 25 mice, only 10 and 5%, respectively, of their body weight was fat (Table II). This represented a specific reduction in body fat of 47 and 77% in line 23 and line 25, respectively, while lean body mass was only marginally reduced by 5–8%. Finally, although body fat represents less than 15% of body weight, it accounted for between 54 and 74% of the reduction in overall body weight in the transgenic mice. Thus, sustained elevation of plasma IGF-II levels is associated with significant changes in body composition.

The mechanism by which body fat is reduced is not clear at the present time. Preliminary measurements of epididymal fat pads have revealed a large reduction in mass in line 23 and line 25 homozygous male mice. Recent work with another transgenic mouse model, in which brown fat is specifically ablated, has demonstrated that those mice become very obese (48). Therefore, it will be important for us to determine whether brown fat is altered in our transgenic mouse models. Small alterations in brown fat may affect the energy balance in our transgenic animals (49). Since line 25 mice have the greatest reduction of body fat they are the most likely candidates for alterations in brown fat or metabolic rate.

The relative difference in fat loss between lines 23 and 25 could be due to a large number of factors which control whole body metabolism. As already mentioned, the absolute amount of brown fat and basic metabolic rate may be different in these two lines. Furthermore, since the IGF-II levels in line 25 and line 23 are significantly different, it is likely that the insulin-like effects of IGF-II on uptake of glucose in skeletal muscle, or lipolysis in adipose tissue, may be different in the two lines. Our initial data on levels of IGF binding proteins in serum suggest a greater elevation of IGFBPs in line 23 mice than line 25 mice. This raises the additional possibility that IGFBPs may alter the endocrine effects of IGF-II in line 23 to a different degree than in line 25. Whether IGFBPs can differentially alter...
the interaction of IGF-II with different body tissues in vivo (i.e., skeletal muscle versus adipose tissue) is not clear at the present time. Elucidation of the mechanism responsible for altered body composition in these animal models may help in our understanding of the role of IGF-II in man, since IGF-II circulates at a relatively high level in adult humans, and its physiological significance is not currently understood.

The carcinogenesis data suggest a causal role for IGF-II in tumorigenesis in both lines of MUP-IGF-II mice. The frequent occurrence of HCCs in our mice implicates autocrine actions of IGF-II in the liver, which is the main organ expressing the transgene in our mice. Interestingly, we have not observed tumors of the peripheral (scent) gland in male mice which is also a very high expressing organ and we have not observed any distinct pathology in the adrenal gland in which the transgene is expressed at a low level. Lymphoma is the second most frequent tumor in our mice. One possible mechanism is that IGF-II affects the proliferation of hematopoietic cells in the spleen and thymus, and thus affects the size of the precursor cell population susceptible to malignant transformation. This hypothesis is supported by in vitro studies which demonstrate that IGF-II can affect the growth of cells in several hematopoietic lineages (50–52). The absence of kidney tumors in our mice is notable since IGF-II is highly overexpressed in Wilms' tumors (28). Inasmuch as the transgene is not expressed in the kidneys of our mice, this result suggests that IGF-II supplied in an endocrine manner to the kidney is not sufficient to induce pathogenic effects. Therefore, it may be necessary for IGF-II to function in an autocrine mechanism during the development of Wilms' tumor.

Several possibilities for the cellular mode of action of IGF-II include 1) subtle changes in the rate of cell proliferation in various organs which, over the life of the animal, cause specific cells in those organs to become initiated and progress to malignant transformation; 2) a decrease in the rate of cell death or interfering with apoptosis, thus allowing cells a longer lifetime in which to acquire the complex set of genetic lesions necessary for immortalization and malignant transformation; and 3) enhancement of the growth of malignant tumors once they arise spontaneously in older mice. The third mechanism would provide a selective growth advantage to any type of early tumor which could respond to IGF-II, presumably, by activating signal transduction pathways which function through the insulin or IGF-I receptors.

Overall, the long latent period before an increase in tumor frequency is observed strongly suggests that IGF-II functions primarily as a tumor promoter or tumor progression factor in vivo; however, a role as a rather weak tumor initiator cannot be ruled out. Further studies on the mechanism of tumorigenesis in our mice may lead to the development of treatment modalities which interfere with IGF-II action and suppress the growth of a wide variety of neoplasms in man.

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