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Transgenic Mice Expressing Human Fibroblast Growth Factor-19 Display Increased Metabolic Rate and Decreased Adiposity

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The fibroblast growth factors (FGFs), and the corresponding receptors, are implicated in more than just the regulation of epithelial cell proliferation and differentiation. Specifically, FGF23 is a regulator of serum inorganic phosphate levels, and mice deficient in FGF receptor-4 have altered cholesterol metabolism. The recently described FGF19 is unusual in that it is nonmitogenic and appears to interact only with FGF receptor-4. Here, we report that FGF19 transgenic mice had a significant and specific reduction in fat mass that resulted from an increase in energy expenditure. Further, the FGF19 transgenic mice did not become obese or diabetic on a high fat diet. The FGF19 transgenic mice had increased brown adipose tissue mass and decreased liver expression of acetyl coenzyme A carboxylase 2, providing two mechanisms by which FGF19 may increase energy expenditure. Consistent with the reduction in expression of acetyl CoA carboxylase 2, liver triglyceride levels were reduced. (*Endocrinology* 143: 1741–1747, 2002)

THE INCIDENCE OF obesity and type II diabetes is increasing with a consequent increase in the associated mortality and morbidity (1, 2). Increased adiposity develops when absorbed nutrients (metabolizable energy) exceed daily requirements and are stored, for the most part, as triglycerides. A transient increase in energy intake is usually balanced by an increase in resting energy expenditure, resulting in a relatively stable body weight (3, 4). However, weight gain and obesity will result if the increase in energy intake exceeds requirements, and any increase in resting energy expenditure is not fully able to compensate. Pharmacological approaches to the treatment of obesity could impact caloric intake or energy expenditure.

Energy expenditure occurs as a result of processes that allow for work, growth, reproduction, heat, and maintenance of homeostasis. Some aspects of energy expenditure can be regulated, and several hormones that contribute to this regulation have been identified. Thyroid hormone increases metabolic rate or energy expenditure by increasing the activity of several metabolic pathways (for review, see Ref. 5). Leptin ultimately activates sympathetic outflow from the hypothalamus and produces an increase in activity and mass of the brown adipose tissue (BAT) (6, 7). Whether this is the sole, or principal, means by which leptin increases energy expenditure has not been resolved.

Metabolic pathways that can operate in a cyclic pattern have the potential to be energetically very costly, and the appropriate regulation and compartmentalization of these pathways can have significant effects on energy utilization. For example, hepatocytes are capable of both synthesizing and oxidizing fatty acids, but multiple regulatory steps largely prevent this wasteful cycle. One of the more important of these steps governs the entry of fatty acids, via carnitine palmitoyl transferase 1 (CPT1), into the mitochondria (8, 9). The activity of CPT1 is regulated by malonlyl coenzyme A (CoA), and the importance of this pathway for regulating energy expenditure has been convincingly demonstrated in experiments in mice with a mutation in the gene encoding the mitochondrially associated form of acetyl CoA carboxylase (ACC2). These mice appear to be unable to synthesize the malonyl CoA required to inhibit CPT1. Under these conditions, there is an increased metabolic rate and a resistance to diet-induced obesity (10).

The fibroblast growth factor (FGF) proteins have been primarily associated with fibroblast and epithelial cell proliferation, and pattern formation (for review, see Refs. 11 and 12). More recently, however, it appears that some of the newly described FGFs may be more important for maintaining physiological homeostasis. Thus, FGF23 is an important regulator of serum inorganic phosphate levels (13). In addition, mice with a deficiency in FGF receptor-4 (FGFR4) have altered expression of genes, such as cholesterol-7-a-hydroxylase (Cyp7a) and 3-hydrox3-methylglutaryl-CoA reductase (HMG reductase), which are important for cholesterol and bile acid homeostasis (14). The recently described human FGF19 is unusual in that it has no detectable mitogenic activity and binds to only one of the known FGF receptors (FGFR4) (15). Here, we report that transgenic mice expressing human FGF19 had an increased metabolic rate and de-

Abbreviations: ACC, Acetyl CoA carboxylase; ACC1, acetyl CoA carboxylase 1; ACC2, mitochondrially associated form of acetyl CoA carboxylase; BAT, brown adipose tissue; CoA, coenzyme A; CPT, carnitine palmitoyl transferase; Cyp7a, cholesterol-7-a-hydroxylase; FGF, fibroblast growth factor; FGFR, FGF receptor; GTT, glucose tolerance test; HMG CoA reductase, 3-hydrox3-methylglutaryl-CoA reductase; IST, insulin suppression test; UCP, uncoupling protein.

creased adiposity and two potential mechanisms were identified.

Materials and Methods

Animals

All protocols were approved by an Institutional Use and Care Committee. Unless otherwise noted, mice were maintained on standard lab chow in a temperature and humidity controlled environment. A 12-h (1800 h/0600 h) light cycle was used.

Transgenic mice

The human FGF19 cDNA (15) was ligated 3′ to the pRK splice donor/acceptor site that was preceded by either the myosin light chain (16) or the metallothionein promoters (17). The FGF19 cDNA was also followed by the splice donor/acceptor sites present between the fourth and fifth exons of the human GH gene (18). The entire expression fragment was purified free from contaminating vector sequences and injected into one-cell mouse eggs derived from FVB X FVB matings. FGF19 transgenic mice were identified by PCR analysis of DNA extracted from tail biopsies. All transgenic mouse experiments used the inbred (FVB) transgenic mice, and the controls were nontransgenic littermates.

Indirect calorimetry

Oxygen consumption was measured in a Columbus Instruments Oxymax open circuit calorimeter (Columbus, OH).

Diet

Standard mouse chow was Purina 5010 (Harlan Teklab, Madison, WI). The high fat (58% kJ fat) and low fat (10.5% kJ fat) isocaloric diets were based on the diets described by Surwit and colleagues (19) and were purchased from Research Diets (New Brunswick, NJ).

Assays

Insulin and leptin were assayed by ELISA kits (Crystal Chem, Chicago, IL). Glucose was assayed either by LIFESCAN Fast Take glucose meter (Lifescan, Inc., Milpitas, CA) or the glucose oxidase method. All other hormones and serum metabolites were assayed by Anilytics, Inc. (Gaithersburg, MD). Bomb calorimetry of the food and feces for the metabolizable energy calculations was performed by Anilytics, Inc. Fat content in the muscle and liver was assayed using the extraction procedures of Folch *et al.* (20) and an enzymatic triglyceride reagent kit (Sigma, St. Louis, MO). Real time quantitative PCR using 100–500 ng total RNA was performed as described (21). Expression for each gene was determined, from the Ct values, relative to the expression of the ribosomal protein gene RPL19. The mean relative expression for the control mice set to 1.0 and the expression in the transgenic mice calculated relative to the control mice. Primers and probe sequences are available on request.

Glucose metabolism

Glucose tolerance tests (GTT) were performed by injecting each mouse with the same amount of glucose (35 mg glucose ip). As the transgenic mice weighed less than the control mice, the transgenic mice received more glucose as a function of body weight (approximately 1.4 mg glucose/g body weight) than the wild-type mice (approximately 1.2 mg glucose/g body weight). This relative increase in glucose dosing in the transgenic mice emphasizes the lower blood glucose levels seen in these mice. Conversely, insulin suppression tests (ISTs) were performed by injecting each mouse with 0.3 IU insulin/kg iv. Thus, in this case the transgenic mice received less total insulin than the wild-type controls, and again emphasizes the lower glucose levels seen in the transgenic mice. For both the GTT and the IST, whole blood glucose was measured at the indicated times using a LIFESCAN Fast Take glucose meter.

Temperature and activity

Core body temperature was monitored telemetrically by ip implanted Physiotel body temperature transmitter devices (Data Sciences International, St. Louis, MO). Activity was monitored via the analysis of the frequency with which the mice broke infrared beams that were placed one per inch (x- and y-axes).

Analysis

Unless otherwise noted all data are presented as the means plus and minus the standard deviations. Comparisons between transgenic and wild-type mice were made using an unpaired *t* test. Oxygen consumption is expressed as a function of body mass raised to the 0.75 power (22–24).

Results

We have asked whether further insights into the biology of FGF19 could be ascertained from the phenotype of FGF19 transgenic mice. Transgenic mice expressing FGF19 under the control of the myosin light chain promoter were generated and, as has been reported previously for transgenic mice with this promoter (16), the mice described here predominantly express the transgene in the muscle (data not shown). Multiple founder animals and two independent lines demonstrated the phenotype described below. Transgenic mice with FGF19 under the control of the metallothionein promoter also had a phenotype comparable to that described below (data not shown). As similar results were obtained from multiple transgenic mice using two different promoters, the phenotype described below appears to result from expression of FGF19 rather than an insertion event.

On standard lab chow, the transgenic mice weighed less than the control mice (Fig. 1A). The difference was significant at 5 d for the male mice (Fig. 1A) and 31 d for the female mice (data not shown). The largest differences (both absolute and proportional) were obtained at 3–4 wk of age for both sexes. Analysis of body composition revealed that a primary reason for the decreased body weight was a significant reduction in fat content. This was seen with both total body composition analysis (Fig. 1B) and fat pad weights (Fig. 1C). There was no significant reduction in either protein or ash content (Fig. 1B) and (with the exception of the BAT, Fig. 4) the other major

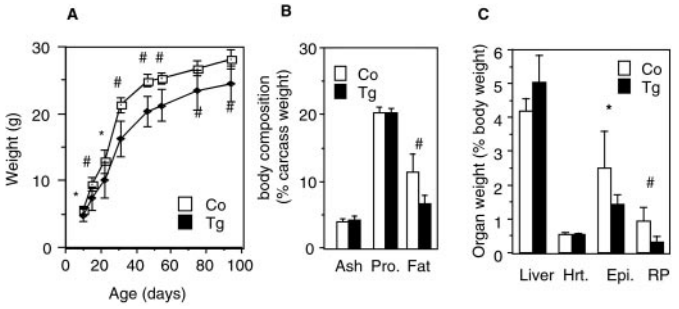

FIG. 1. MLC-FGFG19 transgenic mice weigh less and have less fat. A, Growth curves for control and transgenic male mice (means ± SD; n = 9–10/group. B, Body composition of male control and FGF19 transgenic mice (means ± SD; 12 wk of age, n = 6/group). C, Liver (Liv), heart (Hrt), and fat pad (Epi, epididymal; RP, retroperitoneal) weights of male control and FGF19 transgenic mice (means ± SD; 16 wk of age, n = 11–16/group). □, Control; ■, transgenic. For A–C, *, $P < 0.05$; #, $P < 0.01$.

organs were proportional to body weight (Fig. 1C and data not shown).

The decrease in body weight did not appear to be caused by poor nutrient absorption. In fact, total food intake was significantly greater in the transgenic mice compared with the controls (Fig. 2A). Absorption was also not impaired. Calorimetric analysis of the food and feces indicated that the metabolizable energy for the transgenic mice (measured as protein and fat intake, or as energy content) was significantly greater for the transgenic mice (Fig. 2B).

Mechanism

The mechanism by which the transgenic mice can maintain decreased fat content while continuing to have a greater energy intake was explored by measuring metabolic rate. The data shown in Fig. 3A (for two independent lines of transgenic mice) demonstrated that both male and female transgenic mice had a higher VO_2. This was most obvious during the night when mice are active and feeding. The effect of FGF19 was diminished during the light cycle. There was, however, no detectable difference in metabolic rate between fasted transgenic mice and fasted controls. There was no obvious change in respiratory quotient in the FGF19 transgenic mice (data not shown). The increase in metabolizable energy (Fig. 2, A and B) with no increase in growth or storage (Fig. 1A) and the increase in VO2 (Fig. 3A) with no change in respiratory quotient, pointed to an increase in energy expenditure in the transgenic mice. This increase was reflected in a very small (approximately 0.2 C) increase in temperature as the mice approach the nadir of the diurnal rhythm (Fig. 3B). The three lowest readings taken in the control mice, and the corresponding values in the transgenic mice, are shown separately in the right panel of Fig. 3B. Activity analysis using a two-dimensional beam-breaking format indicates that there was no significant difference between the transgenic and the control mice with respect to coarse motor activity (Fig. 3C). Other than the decreased adiposity, the FGF19 transgenic mice were relatively normal. They were fertile and their hematological profile and serum electrolytes were normal (data not shown). Histological analysis indicated that all major organs were normal with the exception of the liver. In the older (greater than 5 months) transgenic mice, there were dysplastic changes in the liver (Nicholes, K., S. Guillet, E. Tomlinson, K. Hillan, B. Wright, G. D. Frantz, T. A. Pham, L. Dillard-Telm, S. P. Tsai, J.-P. Stephan, J. Stinson, T. Stewart, and D. M. French, manuscript in preparation).

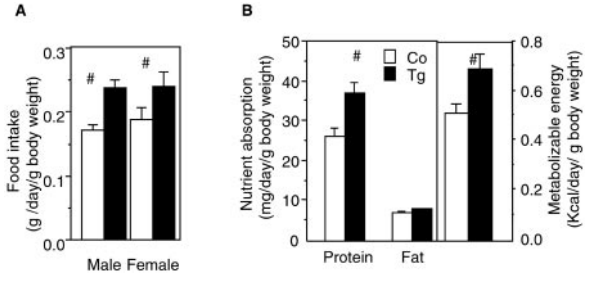

FIG. 2. MLC-FGFG19 transgenic mice eat more than the control mice. A, Total food intake (means ± SD; 9 wk of age, n = 4/group; B, metabolizable energy analysis of male control and FGF19 transgenic mice (means ± SD; 9 wk of age, n = 8/group). □, Control; ■, transgenic. For A and B, #, $P < 0.01$.

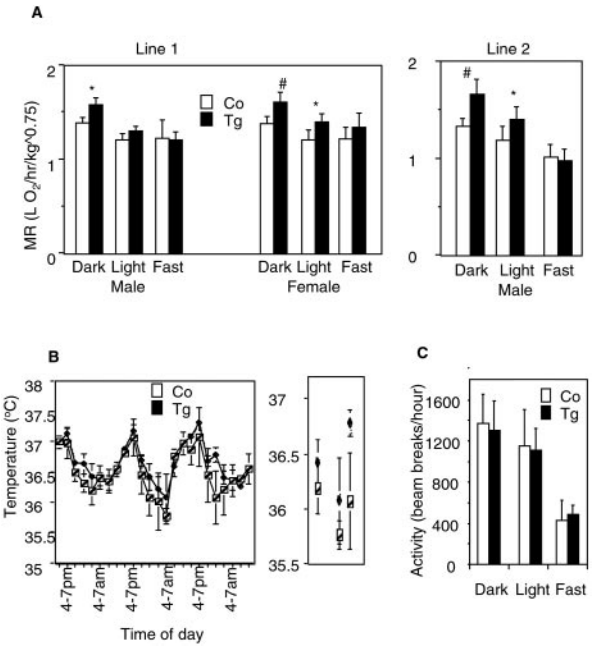

FIG. 3. There is an increase in metabolic rate in the FGF19 transgenic mice. A, Metabolic rate as measured by oxygen consumption in two independent lines of transgenic mice (means ± SD; n = 4/group, 9 wk of age). B, Body temperature in transgenic and control males (n = 4–5/group, 10 wk of age). The *right panel* presents separately the lowest values taken for each of the three cycles shown. C, An analysis of coarse motor activity (males, n = 6–7/group, 10 wk of age). □, Control; ■, transgenic. For A–C, *, $P < 0.05$; #, $P < 0.01$.

As leptin is a circulating hormone (25) that increases fat loss independently of decreased food intake (26), serum levels of this hormone were measured. We found that leptin was actually reduced in the FGF19 transgenic mice (see below). Thyroid hormone levels were not increased in the transgenic mice (Table 1), and there was no thyroid hyperplasia (data not shown). Both GH and IGF-1 have also been implicated in maintaining body composition (27, 28). GH levels were not obviously altered (data not shown), whereas total IGF-1 was reduced (Table 1). Corticosterone was also not altered, but there was a reduction in glucagon (Table 1).

In mice, BAT has been implicated in maintaining body composition. Most convincingly, mice with genetically reduced BAT [uncoupling protein (UCP)-DTA transgenic mice] become obese (29). In the FGF19 transgenic mice, the interscapular BAT depot was significantly enlarged (Fig. 4A). The BAT in the FGF19 transgenic mice appeared histologically normal (data not shown), suggesting that the increased relative size of the BAT may also translate into a relative increase in the activity of this organ. We also investigated the expression of the UCPs in the BAT. There was an approximately 4-fold increase in the expression of UCP2 (Fig. 4B). The expression of UCP 1, 3, 4, and 5 was not different in the transgenic mice compared with the controls.

For several reasons (see *Discussion*), we consider the liver as major target organ for FGF19. Thus, we have measured the mRNA levels, in the liver, of several metabolically important genes (Fig. 5A). We found that the expression of the genes for

TABLE 1. Hormone and metabolite values in the control and FGF19 transgenic mice

A.	Triglycerides (g/liter)	Cholesterol (mmol/liter)	Albumin (g/liter)	Creatinine (mol/liter)	ALT (U/liter)
Control (F)	2.6 ± 0.2	3.2 ± 0.1	37 ± 5	31 ± 5	47 ± 3
Transgenic (F)	2.2 ± 0.2[a]	2.5 ± 0.2[b]	37 ± 3	29 ± 4	52 ± 10
Control (M)	2.4 ± 0.2	4.1 ± 0.2	32 ± 1	28 ± 4	48 ± 3
Transgenic (M)	2.1 ± 0.2[a]	2.6 ± 0.2[b]	35 ± 1[b]	33 ± 4	68 ± 8[b]
B.	T_3 (nmol/liter)	T_4 (nmol/liter)	Glucagon (ng/liter)	Corticosterone (nmol/liter)	IGF-1 (g/liter)
Control (F)	1.2 ± 0.1	40 ± 0.4	41 ± 5	850 ± 270	720 ± 70
Transgenic (F)	1.2 ± 0.1	40 ± 0.1	33 ± 3[a]	1070 ± 360	410 ± 50[b]
Control (M)	1.2 ± 0.1	36 ± 5	42 ± 2	510 ± 90	680 ± 60
Transgenic (M)	1.1 ± 0.1[a]	35 ± 0.3	39 ± 2[a]	490 ± 360	420 ± 40[b]

Data are from 10-wk-old mice on regular chow, fasted for 4 h; n = 4–5.
[a], $P < 0.05$; [b], $P < 0.01$.
F, Female; M, male.

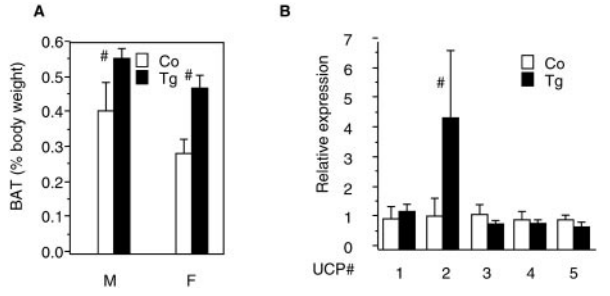

FIG. 4. The BAT pad weights are increased in the FGF19 transgenic mice. A, BAT weights in the FGF19 transgenic mice and controls for both males (M, means ± SD; 9 wk of age, n = 4–5/group) and females (F, means ± SD; 9 wk of age; F, n = 6/group). B, Relative expression of UCP genes in the BAT of the transgenic and control mice monitored by real time quantitative PCR (means ± SD; males, n = 5/group; 12 wk of age). For A and B, *, $P < 0.05$; #, $P < 0.01$.

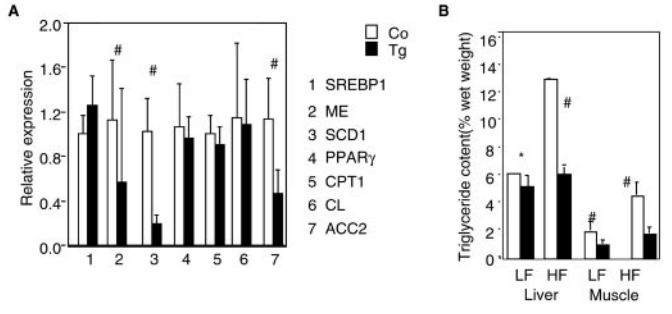

FIG. 5. FGF19 leads to changes in the expression of metabolically relevant genes in the liver. A, Relative expression of the genes encoding sterol response element binding protein 1 (SREBP1), malic enzyme (ME), stearoyl CoA desaturase 1 (SCD1), PPARγ, CPTI, citrate lyase (CL), and ACC2 in the livers of transgenic mice and the appropriate control mice. B, Triglyceride levels in the liver and muscle of mice fed either of a low fat (LF) or high fat (HF) diet. □, Control; ■, transgenic. For A and B; n = 6/group, *, $P < 0.05$; #, $P < 0.01$.

two important lipogenic enzymes (malic enzyme and stearoyl CoA desaturase) was significantly reduced in the transgenic mice (Fig. 5A). In addition, we found that there was a significant reduction in the expression of ACC2 (Fig. 5A). This enzyme appears to be a major regulator of the rate of entry of fatty acids into the mitochondria and mice genetically deficient in this enzyme have an increase in metabolic rate and a decrease in adiposity (10). We found no change in the expression of the insulin receptor, insulin receptor substrates 1 and 2, phosphotyrosine phosphatase 1B, CPT1, acetyl CoA carboxylase 1 (ACC1), and acyl CoA dehydrogenase, long (data not shown).

To investigate whether the decrease in ACC2 is associated with metabolically relevant consequences, we measured liver triglyceride levels. Consistent with an ACC2 regulated effect on fatty acid oxidation rates, we found that mice fed either a low or a high fat diet had significantly reduced liver triglyceride levels (Fig. 5B). The muscles of the transgenic mice also had reduced triglyceride. We did not, however, see any change in expression of muscle ACC2 (data not shown); thus, the lower muscle triglyceride may be a secondary consequence of a reduction in total body fat.

FGF19 and diet induced obesity and diabetes

As the chow fed FGF19 transgenic mice have decreased fat content (Fig. 1), we investigated the effect of FGF19 expression on the response to a high fat diet (30). For both male (Fig. 6A) and female (data not shown) control mice (*open symbols*), there is a significant effect ($P < 0.05$) of the diet at 6 wk of age and older. In contrast, there is no discernible effect of the high fat diet on the transgenic mice (*filled symbols*, $P > 0.1$). After 12 wk on the high fat diet, the mice were killed and the weights of the internal organs determined. The most striking differences were in the white adipose depots (Fig. 6B). The fat pads from the transgenic mice (both males and females) weighed significantly less than the corresponding fat pads from the control littermates. Consistent with the lower adiposity (31), leptin levels in these high fat fed mice were also lower (Fig. 6C). As was found for the chow fed mice, the relative size of the liver (Fig. 6B), and other major internal organs (data not shown) were not different in the transgenic mice compared with the controls.

After 12 wk on the defined diets, glucose metabolism was investigated using a glucose tolerance test (Fig. 7A). There is a significant ($P < 0.05$) effect of the diet in the control mice at all time points except for the final (180 min) value. For the transgenic mice, there is an apparent effect ($P < 0.05$) of the diet only at the 30-min time point. At all time points, the glucose levels in the transgenic mice were significantly less than the control mice (within diet comparisons). Further, the glucose levels in the high fat fed transgenic mice were lower than the chow fed control mice for all but two time points (15 and 30 min).

We also performed an IST. An injection of 0.3 IU insulin/kg body weight led to significantly lower glucose levels

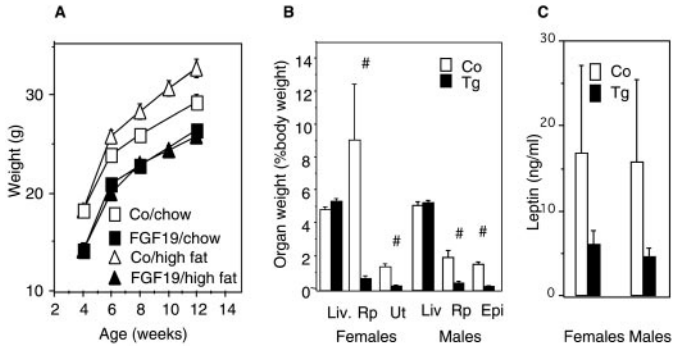

FIG. 6. MLC-FGFG19 mice are resistant to the adipogenic effects of a high fat diet. A, Growth curves of male mice (n = 6–12/group) on either chow (*squares*) or a high fat diet (*triangles*). Data are presented as means ± SEM. *P* values have been omitted for clarity. B, Liver (Liv) and fat pad (Rp, retroperitoneal; Ut, uterine; Epi, epididymal) weights in female and male mice that had been on a high fat diet from the age of 4 through 16 wk of age. (Means ± SEM; 16 wk of age, n = 9–11/group). C, Leptin levels in female and male mice that had been on a high fat diet from the age of 4 through 16 wk of age. (Means ± SD; 16 wk of age, n = 9–11/group).

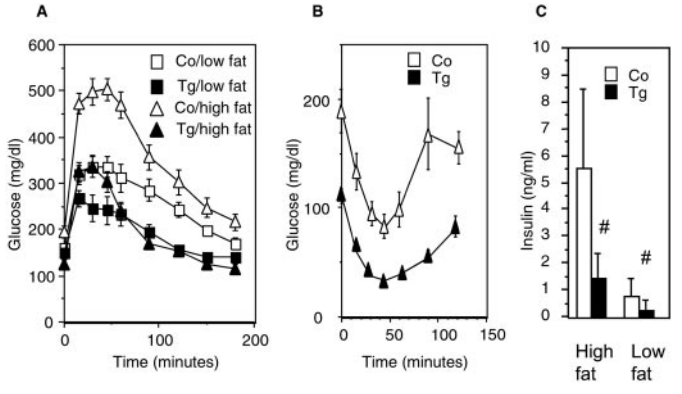

FIG. 7. MLC-FGFG19 mice are resistant to the diabetogenic effects of a high fat diet. A, Glucose tolerance tests in male mice on either a high fat (*triangles*) or a chow diet (*squares*). Data are presented as means ± SEM. B, Glucose levels in high fat fed male FGF19 transgenic mice and controls in response to injected insulin. C, Insulin levels in male mice fed a high or low fat diet for 12 wk. Means ± SD; 16 wk of age, n = 7–11/group). For clarity, the statistical symbols in A and B have been omitted. For C, #, $P < 0.01$.

in the transgenic mice compared with their control siblings [shown for males in Fig. 7B; females have a similar response (data not shown)]. In these experiments there was also a greater proportional decrease. The mean glucose level in the control mice at 45 min was 43 ± 10% (mean ± SD) of the zero time point value whereas for the transgenic mice the comparable values were 31 ± 9 ($P = 0.014$). We also measured circulating insulin levels (Fig. 7C). The high fat diet led to a significant ($P < 0.05$) increase in random fed insulin levels in the control mice. The effect of the diet on the transgenic mice was not statistically significant ($P > 0.05$). On both diets the transgenic mice had significantly lower insulin levels than the control mice ($P < 0.01$ for both diets). These findings—the decreased glucose excursion seen in the GTT, the lower glucose levels attained in the IST, and the lower insulin levels—all point to a significant increase in insulin sensitivity in these transgenic mice.

Discussion

Several obvious changes occur in transgenic mice expressing FGF19—increased metabolic rate, decreased adiposity, increased food intake, and increased insulin sensitivity. As there was no increase in those hormones most often associated with an increase in metabolic rate (leptin, IGF-1, GH, T_3), the FGF19-induced increase in metabolic rate may be a direct effect of FGF19 rather than mediated by intermediary hormones. Possible mechanisms by which FGF19 could induce an increase in metabolic rate are discussed below.

As we have been unable to detect the expression of FGFR4 in adipocytes that have been extensively purified free from contaminating stromal cells (data not shown), it is likely that the decrease in adiposity is secondary to the increased metabolic rate (32).

The increase in food intake in the transgenic mice is also probably indirect. Decreased leptin (25, 33), insulin (34, 35), and CNS glucose utilization (36) all induce feeding behavior in mice, and circulating levels of leptin, insulin, and glucose are decreased in the transgenic mice.

The increase in insulin sensitivity in the transgenic mice may be an indirect consequence of the decrease in adiposity and tissue triglycerides. The relationship between insulin sensitivity and obesity (37, 38) and intramyocellular triglycerides (39) is now well established, and the FGF19 transgenic mice had a significant reduction in tissue (muscle and liver) triglycerides as well as the obvious decrease in adipose tissue. The decrease in liver triglycerides may be a direct consequence of the decreased ACC2 expression and the increased fatty acid oxidation that occurs with decreased ACC2 levels (10). Consistent with an indirect effect on insulin sensitivity through decreased adiposity, we did not detect differences in the expression of a number of genes implicated in mediating insulin signaling.

Mechanisms

For several reasons, we favor the possibility that the liver is a primary target for FGF19. First, the liver is a central organ in regulating carbohydrate and lipid metabolism and is responsible for a disproportionate amount (relative to body mass) of the standard metabolic rate (40). Further, in rats that have undergone a partial hepatectomy, there is a substantial loss of diet induced thermogenesis (41). Second, FGFR4, a reported receptor for FGF19 is highly expressed in liver (15) and hepatocytes (42). Although FGFR4 appears to be at least one receptor for FGF19, an altered metabolic rate was not reported in FGFR4 knockout mice (43); they do, however, have altered cholesterol metabolism (14), suggesting a role for this receptor in regulating lipid metabolism. Third, circulating levels of two liver markers (albumin and alanine amino transferase) were increased (males only, Table 1). Fourth histological changes were found in the livers of the older transgenic mice (Nicholes, K., S. Guillet, E. Tomlinson, K. Hillan, B. Wright, G. D. Frantz, T. A. Pham, L. Dillard-Telm, S. P. Tsai, J.-P. Stephan, J. Stinson, T. Stewart, and D. M. French, manuscript in preparation), as has been reported for mice with altered metabolism caused by deletions in the genes encoding Glut 2 (44) or the insulin receptor (45).

An increased metabolic rate could be achieved through an

increased flux of fatty acids through the mitochondrial β oxidation pathway. This process is normally regulated by both a mass action effect on a system that may be close to equilibrium and by substrate availability. Substrate availability is controlled by the transport of fatty acids (as carnitine esters) through the CPT1 (46) and the major regulator of CPT1 activity is malonyl CoA (8). Malonyl CoA is synthesized by acetyl CoA carboxylase, which exists in two isoforms (ACC1 and ACC2). The isoform responsible for regulating CPT1 activity is ACC2 (47). Thus, as ACC2 decreases (as occurs in the FGF19 transgenic mice), mitochondrially associated malonyl CoA levels decrease, CPT1 activity increases and there would be increased availability of fatty acids for β oxidation. The importance of ACC2 with respect to fatty acid oxidation has been convincingly demonstrated in mice deficient for ACC2 (10). These mice are lean and do not become obese on a high fat diet. While the level of ACC2 is decreased in the FGF19 transgenic mice to approximately 40% of normal, it is not clear how this translates into malonyl CoA levels. Relevant to this is the observation that, even in the complete absence of ACC2, the level of total liver malonyl CoA was not detectably changed (10). This failure to detect changes in the total pool of malonlyl CoA presumably reflects the activity of ACC1 and the impact on CPT1 being via local (mitochondrial) production of malonyl CoA by ACC2. In contrast to the reduction of ACC2, FGF19 had no detectable effect on the expression of ACC1, the isoform apparently responsible for generating the malonyl CoA that is the precursor for fatty acid synthesis.

Another possible cause of the increased metabolic rate in the FGF19 transgenic mice is a change in the biology of the BAT and the associated UCPs (for review, see Ref. 48). A role for BAT in regulating body composition was demonstrated in mice in which there is genetic reduction of the BAT. These mice develop obesity and hyperphagia (29). We have found that the relative weight of the BAT was increased in the transgenic mice and the tissue appeared normal by both histological appearance and expression of the major uncoupling protein, UCP1. Although the BAT changes seen in the FGF19 transgenic mice were similar to those seen in rodents treated with norepinephrine (49) or with specific β3 adrenergic receptor agonists (32), further analyses are required to determine whether the increase in BAT mass is causally related to the increase in metabolic rate. FGF16 is a mitogen for primary brown adipocytes (50), although the expression of this growth factor did not correlate with the BAT hyperplasia induced by cold exposure. Although FGF19 does not appear to be mitogenic (15), it is possible that this growth factor alters the differentiation of the brown adipocytes.

Mice with a genetically engineered reduction in BAT become obese (29). In contrast, mice deficient in the principal BAT uncoupling protein, UCP1, do not (51), suggesting that other mechanisms (possibly including the other UCPs) are important in the physiological functioning of this organ. In the BAT from the FGF19 transgenic mice, there was a disproportional increase in UCP2. The gene encoding UCP2 was identified as residing in a locus that controls obesity (52), and there is an inverse correlation between expression of this gene correlated with body fat in humans (53). While the normal physiological function of UCPs 2–5 is still unclear, transgenic overexpression of both UCP1 (54) and UCP3 (55) in muscle can prevent obesity.

Two plausible mechanisms for the increase metabolic rate have been described—an increase in BAT mass and a decrease in liver ACC2. How these components are woven together and the relative importance of each in the biology elicited by FGF19 will be of the subject of future studies.

Conclusion

The ability of FGF19 to increase metabolic rate and reduce adiposity is striking considering that few other changes are seen in these transgenic mice. Total body length was relatively unaffected, serum electrolytes were normal, and both males and females were fertile (data not shown). Histological examination of internal organs reveals that, in some transgenic mice that are older than 5 months, there were regions of liver dysplasia, whereas all other tissues were normal (data not shown). The specific effects of FGF19 may be related to the observation that FGF19 interacts only with FGFR4 (15) and that FGFR4 is not able to transmit a mitogenic signal (56). It will be of considerable interest to determine whether the increased metabolic rate and decreased adiposity can be elicited by systemic delivery of a recombinant protein.

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