Combination therapy with acipimox enhances the effect of growth hormone treatment on linear body growth in the normal and small-for-gestational-age rat

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Vickers, M. H., P. L. Hofman, P. D. Gluckman, P. E. Lobie, and W. S. Cutfield. Combination therapy with acipimox enhances the effect of growth hormone treatment on linear body growth in the normal and small-for-gestational-age rat. Am J Physiol Endocrinol Metab 291: E1212–E1219, 2006. —Growth hormone (GH) therapy is often associated with adverse side effects, including impaired insulin sensitivity. GH treatment of children with idiopathic short stature does not lead to an optimized final adult height. It has been demonstrated that FFA reduction induced by pharmacological antilipolysis can stimulate GH secretion per se in both normal subjects and those with GH deficiency. However, to date, no investigation has been undertaken to establish efficacy of combination treatment with GH and FFA regulators on linear body growth. Using a model of maternal undernutrition in the rat to induce growth-restricted offspring, we investigated the hypothesis that combination treatment with GH and FFA regulators can enhance linear body growth above that of GH alone. At postnatal day 28, male offspring of normally nourished mothers (controls) and offspring born with low birth weight [small for gestational age (SGA)] were treated with saline, GH, or GH (5 mg·kg⁻¹·day⁻¹) in combination with acipimox (GH + acipimox, 20 mg·kg⁻¹·day⁻¹) or fenofibrate (GH + fenofibrate, 30 mg·kg⁻¹·day⁻¹) for 40 days. GH plus acipimox treatment significantly enhanced linear body growth in the control and SGA animals above that of GH, as quantified by tibial and total body length. Treatment with GH significantly increased fasting plasma insulin, insulin-to-glucose ratio, and plasma volumes in control and SGA animals but was not significantly different between saline and GH- plus-acipimox-treated animals. GH-induced lipolysis was blocked by GH plus acipimox treatment in both control and SGA animals, concomitant with a significant reduction in fasting plasma FFA and insulin concentrations. This is the first study to show that GH plus acipimox combination therapy, via pharmacological blocking of lipolysis during GH exposure, can significantly enhance the efficacy of GH in linear growth promotion and ameliorate unwanted metabolic side effects.

GROWTH HORMONE (GH) THERAPY of short normal children or children born small for gestational age (SGA) is often associated with unwanted side effects, such as impaired insulin sensitivity and an enhanced susceptibility, for the development of type 2 diabetes (5, 12, 22). Furthermore, GH treatment of children with idiopathic short stature or SGA does not lead to achievement of genetic height potential (9, 15, 31). GH is a lipolytic hormone that induces elevations in free fatty acid (FFA) concentrations, and it is speculated that worsening of insulin sensitivity is a consequence of enhanced omental lipolysis, leading either to alterations in intermediate metabolism or to alterations in membrane characteristics (6, 27, 32). Previous work in GH-deficient adults (35, 36) has shown that prevention of lipolysis by coadministration of GH with FFA regulators can partially prevent the deterioration of insulin sensitivity. This work suggested that GH-induced insulin resistance is mediated by GH-induced lipolysis and that inhibition of lipolysis may result in improved insulin sensitivity. To date, however, no studies have been performed that investigate the effect of coadministration of GH and FFA regulators on enhancement of linear growth.

The present trial utilized acipimox and fenofibrate, two FFA regulators that act as antilipolytic agents via different mechanisms. Acipimox, a potent long-acting nicotinic acid derivative, lowers FFAs and triglyceride levels, reduces lipid oxidation rate, and increases glucose oxidation rate (8, 25, 33). Acipimox can lower plasma insulin concentrations and has been shown to enhance spontaneous GH secretion in obese humans (17) and to considerably enhance GH secretion in response to various secretagogues (3, 18, 21, 23). Acipimox has also been shown (30) to restore the GH response to GH-releasing hormone in elderly subjects. Fenofibrate, a fibric acid derivative, efficiently lowers serum triglyceride levels through mediation of the peroxisome proliferator-activated receptor-α (13, 16).

The objective of the present study was to evaluate the effectiveness of a combination therapy comprising GH and FFA regulators in improving linear growth via pharmacological antilipolysis.

MATERIALS AND METHODS

Animal Model to Induce SGA Offspring

Virgin Wistar rats (age 100 ± 5 days, n = 15/group) were timed mated using a rat estrous cycle monitor to assess the stage of estrus of the animals before the male was introduced. After confirmation of mating, rats were housed individually in standard rat cages containing wood shavings as bedding and free access to water. All rats were kept in the same room with a constant temperature maintained at 25°C and a 12:12-h light-dark cycle. Animals were assigned to one of two nutritional groups: group 1, undernutrition [30% of ad-libitum (SGA)] of a standard diet throughout gestation; and group 2, standard diet ad libitum (controls) throughout pregnancy. Food intake and maternal weights were recorded daily until birth. After birth, pups were weighed and litter size recorded. Pups from undernourished mothers were cross-fostered onto dams that received ad libitum feeding of a standard diet throughout gestation; and group 2, standard diet ad libitum (controls) throughout pregnancy. Food intake and maternal weights were recorded daily until birth. After birth, pups were weighed and litter size recorded. Pups from undernourished mothers were cross-fostered onto dams that received ad libitum feeding.
weaning, all offspring were fed a standard control diet for the duration of the study (Diet 2018; Harlan Teklad, Oxon, UK). At postnatal day 28, male offspring from the two groups of dams, control offspring and offspring from undernourished mothers (SGA), were weight matched (\( n = 10 \)/group) and received either saline, recombinant bovine GH (at a dose of 5 mg·kg\(^{-1}·\text{day}^{-1}\)), GH, and acipimox (GH + acipimox, 20 mg·kg\(^{-1}·\text{day}^{-1}\)) or GH and fenofibrate (GH + fenofibrate, 30 mg·kg\(^{-1}·\text{day}^{-1}\)) for 40 days. Saline or GH was administered by subcutaneous injection at 0800 and 1700 using a split-dose protocol, as described previously (41). Acipimox (Pfizer) and fenofibrate (Sigma, St. Louis, MO) were administered via oral gavage at 0800 (volume 0.5 ml in 5% gum arabic solution). Animals not receiving acipimox or fenofibrate were gavaged with gum arabic solution.

Body weight and food and water intakes of all offspring were measured daily throughout the experiment. Systolic blood pressure was measured at day 65 using tail cuff plethysmography (IITC Life Science, Woodland Hills, CA). At the end of the treatment period, rats were fasted overnight and killed by halothane anesthesia followed by decapitation. Blood was collected into heparinized vacutainers and stored on ice until centrifugation and removal of supernatant for analysis. All animal work was approved by the Animal Ethics Committee of the University of Auckland.

**Laboratory Methods**

**Bone scanning.** Tibias were isolated and stored in 10% neutral buffered formalin. Tibial length was assessed using peripheral quantitative computed tomography using a Stratec XCT-2000 scanner (Stratec, Germany). Total bone density incorporates both cortical and trabecular bone mass.

**Plasma analysis.** IGF-1 in rat blood plasma was measured using an IGF-binding protein-blocked radioimmunoassay (RIA), as described previously (40). The half-maximally effective dose was 0.1 ng/tube, and the intra- and interassay coefficients of variation were <5 and <10%, respectively. Fasting plasma insulin and leptin were measured by RIA, as described previously (19, 39). Fasting plasma glucose concentrations from samples taken at the time of death were measured using a YSI Glucose Analyzer (Model 2300, Yellow Springs Instruments, Yellow Springs, OH). Blood plasma FFAs and triglycerides were measured by diagnostic kit (Boehringer-Mannheim no. 1383175 and Sigma no. 337, respectively). All other plasma analytes were measured by a BM-Hitachi 737 analyzer.

Statistical analyses were carried out using StatView (SAS Institute, Cary, NC) and SAS software packages. Differences between groups were determined by factorial ANOVA (prenatal background and treatment as factors) followed by Bonferroni post hoc analysis. Criteria analysis within StatView was also utilised to test for within-group treatment effects and interactions (control and SGA grouping). Data are shown as means \( \pm SE; n = 10 \)/group.

**RESULTS**

**Animal Model to Induce SGA Offspring**

Maternal undernutrition resulted in fetal growth retardation, reflected by decreased body weight at parturition in the offspring from SGA dams (control males 6.1 \( \pm 0.49 \) g, SGA 4.2 \( \pm 0.6 \) g, \( P < 0.001 \)). Litter size was not different between the two groups (control 13.3 \( \pm 0.4 \), SGA 12.8 \( \pm 1.0 \)). Nose-anus (NA) and nose-tail (NT) lengths were shorter at birth in SGA offspring compared with control offspring (NA: control males 49.3 \( \pm 2.43 \) mm, SGA males 44 \( \pm 3.0 \) mm; NT: control males 65.9 \( \pm 2.8 \) mm, SGA males 58 \( \pm 4.1 \) mm, \( P < 0.001 \) for both lengths). At the commencement of treatment, SGA offspring were lighter than control animals (\( P < 0.001 \)), and total body weights remained lower in SGA offspring for the remainder of the study.

**Treatment Effects**

Body weight gain (gain in grams) was increased in all treatment groups (\( P < 0.001 \)) compared with saline. There were no differences in absolute body weight gain between animals treated with GH and the combination therapies (Fig. 1). GH-plus-acipimox-treated animals had a greater body weight gain than GH-plus-fenofibrate-treated animals. Compared with GH alone, control animals treated with GH plus acipimox showed a gradual divergence from GH-alone animals in body weight gain. However, the effect of the GH-plus-acipimox and GH-plus-fenofibrate combination treatments in control animals was diminished by postnatal day 57 compared with GH treatment alone. In SGA animals, GH-plus-fenofibrate-treated animals showed a marked initial increase in weight gain compared with GH-treated animals, but this effect started to diminished after 2 wk of cotherapy, and by the end of the trial these animals were growing at a significantly slower rate than GH-treated animals (Fig. 2). However, SGA animals

![Fig. 1. Body growth curves after 40 days of treatment with either saline, growth hormone (GH), GH + fenofibrate, or GH + acipimox. All groups vs. saline \( P < 0.001 \). There were no significant differences in absolute body weight gain between animals treated with the GH or GH combination therapies; \( n = 10 \)/group.](http://ajpendo.physiology.org/Downloadedfrom)
treated with GH plus acipimox showed a slow but positive weight gain increment compared with GH-treated animals that had not abated at the end of the trial.

GH increased tibial length in all treated groups relative to saline-treated controls (Fig. 3A). In addition, GH plus acipimox treatment further significantly enhanced the GH-induced effects on tibial growth ($P < 0.001$). Tibial lengths in the GH-plus-fenofibrate-treated animals were not different from that of GH alone. Total tibial area was reduced in SGA animals and increased in all GH-treated animals (Table 1). NA lengths were increased in all treatment groups and, in addition, were further increased using combination therapy with GH plus acipimox ($P < 0.001$ for GH vs. GH + acipimox; Fig. 3B). Tibial lengths were highly correlated with total body (NA) length ($r^2 = 0.81$, $P < 0.001$). Total bone density (cortical + trabecular) was not altered in any of the treatment groups (data not shown).

Hormone and Metabolite Data

Plasma IGF-I concentrations were increased in GH and GH-plus-acipimox-treated control and SGA animals but were

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Fig. 2. Change in body weight in control and small-for-gestational-age (SGA) animals treated with GH + acipimox or GH + fenofibrate relative to animals receiving GH only. Animals treated with GH + acipimox showed sustained growth increments over animals treated with GH, particularly in SGA offspring. Control animals treated with GH + fenofibrate showed marginal growth improvement over GH treatment. SGA animals showed a marked initial response in weight gain with GH + fenofibrate treatment, but this became significantly reduced compared with that of GH alone at the completion of the trial; $n = 10$/group.

Fig. 3. A: relative tibial growth as assessed by peripheral quantitative computed tomography (pQCT) in animals treated with GH, GH + fenofibrate (GHF), or GH + acipimox (GHA) compared with saline-treated animals. GHA vs. GH/GHF $P < 0.001$; GHF vs. GH not significant; $n = 10$/group. B: relative change in nose-anus (NA) length compared with saline treated controls. $P < 0.001$ for effect of control vs. SGA and treatment; $P < 0.001$ for treatment vs. saline; $P < 0.001$ for GH and GHF vs. GHA; GH vs. GHF not significant.
not significantly increased in the GH-plus-fenofibrate-treated animals (Table 2). There were no differences in fasting plasma insulin levels between the control and SGA animals. Plasma insulin concentrations were increased in GH and GH-plus-fenofibrate-treated animals compared with saline-treated (P < 0.05) animals but were not increased in the GH-plus-acipimox treatment group (Fig. 4). Fasting plasma glucose concentrations were not different between control and SGA animals and were not altered by GH therapy. Plasma glucose concentrations were lower in the GH-plus-acipimox groups compared with GH alone, and there was an overall trend for glucose to be lower in the GH-plus-acipimox group compared with saline controls (P = 0.07). Glucose concentrations in the GH-plus-fenofibrate treatment groups were increased compared with all other treatment groups (Table 2). Overall, fasting plasma glucose concentrations in the present study were elevated, and this may be a reflection of trunk blood collection under light halothane anesthesia. Tail sampling compared with trunk blood in the same animal provided highly correlated glucose measurements (r^2 = 0.94), although tail sampling yields lower overall glucose values (5.5–6.0 mmol/l). The fasting plasma insulin-to-glucose ratios [insulin (ng/ml) divided by fasting glucose (mmol/l)] were not different between control and SGA animals and were not different between saline and GH-plus-acipimox-treated animals. GH and GH plus fenofibrate treatment increased the insulin-to-glucose ratio compared with saline and GH-plus-acipimox-treated animals (P < 0.05; Table 2). There were no statistically significant differences in plasma leptin concentrations between control and SGA animals. Plasma leptin levels were elevated in all GH-treated animals compared with saline-treated animals and animals that received GH plus fenofibrate. Leptin concentrations were significantly different between GH and GH-plus-fenofibrate-treated SGA animals, but this difference was not observed in control animals. There were no differences in leptin concentrations between GH-treated animals and those administered GH plus acipimox. Plasma FFAs were not significantly different between control and SGA animals and were significantly reduced in control and SGA animals treated with GH plus acipimox compared with saline-treated animals and animals treated with GH alone. Interestingly, the GH-plus-fenofibrate combination did not lower FFA concentrations, and they were higher than those treated with GH plus acipimox, which may reflect waning dose efficacy, as demonstrated in Fig. 2. Plasma triglycerides were not different between control and SGA animals, and

Table 2. Hormone, metabolite, and systolic blood pressure measurements in control and SGA animals after 40 days of treatment with either saline, GH, GH plus fenofibrate, or GH plus acipimox

<table>
<thead>
<tr>
<th>Group</th>
<th>IGF-I, ng/ml</th>
<th>FFA, mmol/l</th>
<th>TG, mmol/l</th>
<th>Glycerol, mmol/l</th>
<th>Leptin, ng/ml</th>
<th>Glucose, mmol/l</th>
<th>I/G Ratio</th>
<th>TP, g/l</th>
<th>Albumin, g/l</th>
<th>TC, mmol/l</th>
<th>SBP, mm/Hg</th>
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</thead>
<tbody>
<tr>
<td>Control Saline</td>
<td>794±73</td>
<td>0.78±0.08</td>
<td>1.25±0.2</td>
<td>0.28±0.02</td>
<td>9.4±0.9</td>
<td>8.9±0.2</td>
<td>0.07±0.01</td>
<td>55.8±0.9</td>
<td>39.2±0.7</td>
<td>2.07±0.1</td>
<td>105±1.1</td>
</tr>
<tr>
<td>Control GH</td>
<td>964±10</td>
<td>0.79±0.04</td>
<td>0.86±0.1</td>
<td>0.29±0.02</td>
<td>12.3±1.4</td>
<td>9.0±0.4</td>
<td>0.10±0.02</td>
<td>57.8±0.9</td>
<td>40.1±0.8</td>
<td>3.12±0.2</td>
<td>106±2.3</td>
</tr>
<tr>
<td>Control GH</td>
<td>932±11</td>
<td>0.54±0.11</td>
<td>0.58±0.6</td>
<td>0.20±0.03</td>
<td>10.3±1.7</td>
<td>8.2±0.4</td>
<td>0.05±0.01</td>
<td>59.9±1.2</td>
<td>41.7±0.9</td>
<td>2.90±0.2</td>
<td>107±2.1</td>
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<tr>
<td>Control GH</td>
<td>871±12</td>
<td>0.76±0.04</td>
<td>1.87±0.2</td>
<td>0.31±0.02</td>
<td>10.1±1.5</td>
<td>9.7±0.2</td>
<td>0.09±0.01</td>
<td>64.7±0.9</td>
<td>47.3±0.8</td>
<td>2.69±0.1</td>
<td>103±8.0</td>
</tr>
<tr>
<td>SGA Saline</td>
<td>794±52</td>
<td>0.82±0.08</td>
<td>0.85±0.1</td>
<td>0.26±0.01</td>
<td>8.6±1.4</td>
<td>8.4±0.2</td>
<td>0.07±0.01</td>
<td>59.0±0.7</td>
<td>40.4±0.4</td>
<td>2.13±0.1</td>
<td>122±5.6</td>
</tr>
<tr>
<td>SGA GH</td>
<td>1044±10</td>
<td>0.83±0.06</td>
<td>1.01±0.1</td>
<td>0.28±0.01</td>
<td>12.7±2.0</td>
<td>8.8±0.3</td>
<td>0.10±0.02</td>
<td>61.0±0.9</td>
<td>42.5±0.8</td>
<td>3.34±0.2</td>
<td>107±3.5</td>
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<tr>
<td>SGA GH</td>
<td>1029±15</td>
<td>0.63±0.10</td>
<td>0.64±0.1</td>
<td>0.22±0.02</td>
<td>10.3±1.2</td>
<td>7.9±0.4</td>
<td>0.07±0.01</td>
<td>63.5±0.9</td>
<td>43.8±0.7</td>
<td>2.88±0.1</td>
<td>108±4.1</td>
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<tr>
<td>SGA GH</td>
<td>854±92</td>
<td>0.74±0.08</td>
<td>1.94±0.1</td>
<td>0.28±0.03</td>
<td>7.7±1.0</td>
<td>9.7±0.1</td>
<td>0.11±0.01</td>
<td>67.1±0.8</td>
<td>49.6±0.5</td>
<td>2.60±0.1</td>
<td>109±4.5</td>
</tr>
</tbody>
</table>

Data are mean ± SE and were analyzed by factorial ANOVA; n = 10/group. FFA, free fatty acids; TG, triglycerides; I/G, insulin (in ng/ml) divided by glucose (in mmol/l); TP, total protein; TC, total cholesterol; SBP, systolic blood pressure.
there was no effect of GH treatment on triglycerides compared with saline controls. Triglycerides were lower in GH-plus-acipimox-treated animals compared with all other treatment groups. There were no differences in plasma glycerol between control and SGA animals, although plasma glycerol was decreased in GH-plus-acipimox-treated animals compared with all other treatment groups. Plasma FFAs and free glycerol were strongly associated ($r^2 = 0.63, P < 0.001$).

Total protein concentrations were increased in SGA animals compared with control animals ($P < 0.001$) and were increased in all treatment groups (total protein concentrations in GH + acipimox- and GH + fenofibrate-treated animals were further increased compared with GH alone; Table 2). Plasma albumin concentrations were higher in all SGA animals compared with control animals ($P < 0.0005$). Albumin concentrations were increased in all treated animals and were highest in those animals treated with GH plus fenofibrate ($P < 0.001$ vs. all other treatment groups). GH plus acipimox increased albumin concentrations above that of GH alone ($P < 0.05$). Total cholesterol concentrations were not different between control and SGA animals and were increased in all treatment groups compared with saline controls. However, the increases in cholesterol in the combination groups were diminished compared with those treated with GH alone (GH vs. GH + acipimox, $P < 0.05$; GH vs. GH + fenofibrate, $P < 0.001$).

**Tissue Weights**

Liver weights relative to body weight were not different between control and SGA animals, and GH or GH plus acipimox treatment had no effect on liver weight. Liver weights were increased in control and SGA animals treated with GH plus fenofibrate (Table 1). There were no significant differences between control and SGA animals in relative retroperitoneal fat depots (Fig. 5). Treatment with GH or GH plus fenofibrate reduced retroperitoneal fat mass compared with saline controls. The combination of GH plus acipimox had no effect on retroperitoneal fat mass compared with saline-treated controls.

**Blood Hematocrit**

Blood hematocrits were reduced in GH-treated animals in both control and SGA groups and are a reliable proxy for fluid retention associated with GH therapy (11). Blood hematocrits were higher in the GH-plus-acipimox-treated animals compared with the GH-alone and GH-plus-fenofibrate groups and were not different from that of saline (Table 1).

**Systolic Blood Pressure**

As shown previously, systolic blood pressures were elevated in SGA animals (Table 2) (39). Treatment of SGA offspring
with GH or GH plus FFA sensitizers reduced and normalized systolic blood pressure. This agrees with our previous reports (41) on the anti-hypertensive effects of GH. Systolic blood pressure was normal in control animals, and there was no effect of any treatment.

**DISCUSSION**

GH replacement therapy in SGA children has previously been shown (4, 5, 34) to induce insulin resistance and an enhanced susceptibility for the development of type 2 diabetes. Furthermore, treatment of short normal or SGA children with GH does not always lead to an attainment of an optimal final adult height (9, 15). The present study is the first to report an enhancement of linear growth over GH therapy concomitant with an amelioration of GH-induced increases in plasma volume and fasting plasma insulin concentrations.

Acipimox has been shown to enhance spontaneous GH secretion in obese humans (17) and in response to various secretagogues (3, 18, 21, 23). Acipimox has also been demonstrated to restore GH response to GH-releasing hormone in elderly subjects (30). However, these previous trials have used acipimox after an acute treatment paradigm, with the effects thought to be a transient phenomenon. To date, no long-term efficacy studies of acipimox in conjunction with GH therapy have been performed, and the use of FFA regulators in combination with GH to enhance linear growth in the early life phase has not yet been investigated. The present study was therefore designed to investigate whether combination treatment with GH plus FFA regulators could enhance linear growth above that of GH alone in a well-characterized animal model of SGA in the rat (39, 40).

The precise mechanism by which GH plus acipimox stimulated linear growth above that of GH remains to be elucidated. We have previously used GH in combination with insulin sensitizers (metformin and troglitazone) and observed no effects on height increments despite improvements in insulin sensitivity (Vickers et al., unpublished observations). Thus it is unlikely that the significant linear growth enhancements observed in the present trial with GH and acipimox cotherapy are mediated directly as a result of alterations in insulin sensitivity.

Targeted disruption studies in rodents have indicated that STAT5b, of the seven mammalian STATs, is required for GH-stimulated sexually dimorphic body growth, with male STAT5b−/− mice reduced to the size of female mice (37). Clinically, STAT5b mutation also results in severe GH insensitivity (14). Therefore, one mechanism by which acipimox increases the efficacy of GH would be to potentially increase the STAT5 response to GH. However, measurement of GH-stimulated, STAT5-mediated transcription by use of a STAT5 reporter assay (20) indicates no effect of acipimox, or nicotinic acid, on the ability of GH to stimulate transcriptional activity of STAT5 (Ling and Lobie, unpublished observations). Acipimox is therefore enhancing GH sensitivity by a mechanism other than the central growth promoting JAK2-STAT5 pathway utilized by GH. It may be that acipimox is working through the mechanism by which GH stimulates female and/or male residual postnatal body growth. In any case, the mechanism(s) by which acipimox enhances GH-stimulated growth remains to be determined.

In addition to acipimox-mediated changes in the GH-FFA feedback cycle, acipimox may also have altered GH output through neural pathways via altered activation of dopaminergic neural circuits (17). Plasma IGF-I levels were not different between control and SGA animals, which concurs with previous work in the rodent by Woodall et al. (42) showing that plasma IGF-I levels, although significantly lower at birth, are normalized in early life. Plasma IGF-I levels were elevated to a similar extent in both GH and GH plus acipimox animals but were not markedly increased in GH-plus-fenofibrate-treated animals. This may be a result of a waning dose efficacy with this latter combination. A lack of significant change in IGF-I concentrations in the GH plus acipimox group compared with GH-treated animals in the presence of enhanced growth may reflect either early effects of acipimox on bone growth or differential effects of acipimox on tissues (i.e., bone vs. liver). There were no adverse side effects observed with prolonged combination therapy with GH plus acipimox, as observed in physiological measurements (food and water intake and hormonal/biochemistry analysis). However, GH plus fenofibrate cotherapy resulted in a marked increase in liver weight, although hepatic function did not appear to be compromised using the available plasma indicators of liver function. As expected, GH plus acipimox significantly lowered plasma FFA and triglyceride concentrations. Acipimox is known to exert its antilipolytic effect by lowering the intracellular level of cyclic adenosine monophosphate and thereby inhibiting the activity of the hormone-sensitive lipase (2, 26). Interestingly, FFA suppression was not observed in the animals cotreated with fenofibrate, but this may relate to efficacy with prolonged exposure, as discussed above. A lack of significant differences in FFA levels between control saline and SGA saline vs. control GH and SGA GH, respectively, suggest that factors other than FFAs may also be responsible for reduced insulin sensitivity and in mediating the role of lipolytic blockers on the increased efficacy of GH in growth promotion. However, the lack of GH treatment on changes in FFAs in the present study may have been a consequence of elevations in FFAs after the fasting period. For example, in bovine GH-transgenic mice, differences in FFA concentrations are observed in the fed state, but they are not different from littermate controls in the fasted state (28). Thus a comparison of FFA concentrations in fed saline and GH-treated animals using the present experimental paradigm may help delineate these effects.

The small but significant increase in plasma leptin concentrations was unexpected because short-term GH treatment to rats normally leads to a decrease in plasma leptin. A recent study by Vestergaard et al. (38) reported an increase in plasma leptin concentrations in patients treated with GH and acipimox. It was speculated that changes in hormone-sensitive, lipase-mediated lipolysis may lead to a feedback stimulation of leptin secretion after combination therapy, but this does not explain the rise in plasma leptin in the GH-only-treated animals, which may be related to length of GH exposure.

SGA animals had elevated blood pressure compared with control animals, which concurs with our previous observations (39). Systolic blood pressure was normalized in SGA animals after GH treatment, as shown previously by our laboratory (41), and these effects were not negated by combination therapy. This observation is in accordance with the observa-
tions of Sas et al. (34) showing a normalization of systolic blood pressure in SGA children after GH treatment.

Our hormone and metabolite data concur with those of others (24) showing that GH plus acipimox combination therapy exerts pronounced effects on protein metabolism, including increased protein synthesis, concomitant with a sustained suppression of circulating FFA, glycerol, and triglycerides. In our animal model and in other rodent models utilizing maternal undernutrition, it has previously been shown that impairments in insulin sensitivity occur in postnatal life, but these changes are normally reported in mature adult or aged animals (29). In the present study, we observed no significant differences in insulin or glucose concentrations, but this may relate to the young age of the animals. Our insulin and insulin-to-glucose ratio data concurs with work by Nielsen et al. (25) showing that pharmacological antilipolysis can restore insulin sensitivity during growth hormone exposure. Impaired insulin sensitivity is the major metabolic abnormality with GH treatment. Furthermore, there is a preexisting reduction in insulin sensitivity in SGA children prior to the onset of GH therapy (4, 7). It has been proposed that reduction in insulin sensitivity during recombinant human (rh)GH therapy may be an essential requirement for rhGH-induced growth promotion (10). This proposal is based on changes in insulin sensitivity that occur at puberty and also during rhGH therapy of short normal children (1, 10). In the present study, despite GH plus acipimox treatment ameliorating the GH-induced elevations in fasting plasma insulin and insulin-to-glucose ratios, the linear growth response with combination treatment was still significantly greater than that observed with GH treatment alone. This negates the proposal that insulin resistance is a requirement for GH-induced longitudinal growth.

In summary, GH plus acipimox treatment enhanced linear growth above that of GH alone and ameliorated the diabeticogenic and fluid retentive effects normally associated with GH therapy. These observations were consistent in both SGA animals and those born of normal birth weight. However, the combination of GH plus fenofibrate was not as effectual as GH plus acipimox and was further compromised by the observed hepatomegaly. We observed beneficial metabolic efficacy of GH plus acipimox (including improved insulin sensitivity) over GH monotherapy. This is the first report indicating a means of optimizing linear growth with GH therapy, reducing plasma fluid volume, and normalizing metabolic parameters such as insulin sensitivity. Translation of these results into the clinical setting would be of major relevance for the assessment of treatment paradigms utilizing GH therapy for linear growth promotion in short normal and SGA children.

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GRANTS

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17. Kok P, Buijs M, Kok S, Van Ierssel I, Frolich M, Roelfsema F, Voshol P, Meinders A, and Pijl H. Inhibition of lipolysis negates the proposal that insulin resistance is a requirement for rhGH-induced growth promotion (10). This proposal is based on changes in insulin sensitivity that occur at puberty and also during rhGH therapy of short normal children (1, 10). In the present study, despite GH plus acipimox treatment ameliorating the GH-induced elevations in fasting plasma insulin and insulin-to-glucose ratios, the linear growth response with combination treatment was still significantly greater than that observed with GH treatment alone. This negates the proposal that insulin resistance is a requirement for GH-induced longitudinal growth.

In summary, GH plus acipimox treatment enhanced linear growth above that of GH alone and ameliorated the diabeticogenic and fluid retentive effects normally associated with GH therapy. These observations were consistent in both SGA animals and those born of normal birth weight. However, the combination of GH plus fenofibrate was not as effectual as GH plus acipimox and was further compromised by the observed hepatomegaly. We observed beneficial metabolic efficacy of GH plus acipimox (including improved insulin sensitivity) over GH monotherapy. This is the first report indicating a means of optimizing linear growth with GH therapy, reducing plasma fluid volume, and normalizing metabolic parameters such as insulin sensitivity. Translation of these results into the clinical setting would be of major relevance for the assessment of treatment paradigms utilizing GH therapy for linear growth promotion in short normal and SGA children.

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GRANTS

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