The Growth Factor from Plerocercoid Larvae of the Tapeworm, *Spirometra mansonoides*, Stimulates Growth But Is Not Diabetogenic (42907)

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Abstract. **A factor produced by plerocercoids of the tapeworm *Spirometra mansonoides* is similar to human growth hormone (hGH) in that it stimulates body growth, binds to hGH receptors, cross-reacts with anti-hGH antibodies, and has lactogenic and insulin-like activities. The purpose of this study was to determine whether plerocercoid growth factor (PGF) is similar to hGH in expressing diabetogenic activity in the genetically obese (*ob/ob*) mouse. To determine an effective dose for use in the obese mice, the ability of daily injections of PGF to stimulate growth of phenotypically normal mice of the same strain was assessed in a 10-day weight gain assay. Injections of PGF stimulated a dose-dependent weight gain ($r = 0.83$) and 25 ng eq/day of PGF stimulated a response not significantly different from that produced by 100 μg of bovine growth hormone/day. Diabetogenicity was assessed using fasting blood glucose and glucose tolerance tests in obese mice that had been injected for 3 days with saline, hGH, or PGF. Human growth hormone caused a significant increase ($P < 0.005$) in fasting blood glucose and glucose tolerance of the obese mice was impaired ($P < 0.01$). All of the doses of PGF used to test diabetogenicity in the obese mice were at least twice that required to stimulate a maximal growth response in normal mice, yet none of the doses of PGF increased fasting blood glucose or decreased glucose tolerance. These results show that PGF was a potent growth stimulant but was not diabetogenic.**

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It is well known that chronic administration of high doses of growth hormone (GH) to susceptible animals such as normal cats, dogs, humans, and obese mice produce hyperglycemia, hyperinsulinemia, and insulin resistance (1–7). Anti-insulin/diabetogenic activities are expressed by GH from fish to humans (5) including the 20,000 variant of human growth hormone (hGH) (6). Although it has been suggested that the diabetogenic activity of GH preparations is due primarily to contamination with small pituitary peptides, the fact that highly purified hGH and recombinant DNA-derived hGH are diabetogenic in the human (3) and animals (6, 7) confirms that diabetogenicity is an intrinsic property of the hGH molecule. Furthermore, structure-function studies suggest that there are multiple active sites in the GH molecule and that the multiple activities of GH may be expressed independently (8).

Plerocercoids of the tapeworm, *Spirometra mansonoides* produce a factor which has many actions similar to those of hGH. Like hGH, plerocercoid growth factor (PGF) stimulates growth which is associated with an increase in somatomedin activity (9), has insulin-like activities both *in vivo* and *in vitro* (10–14), displaces ^{125}I-labeled hGH from both somatogenic and lactogenic receptors (15–17), binds to hGH receptors on human IM-9 cells (18), cross-reacts with monoclonal anti-hGH antibodies (19), and is lactogenic in the pigeon crop-sac assay (20).

Although the similarities between PGF and hGH are remarkable, the differences may be more important. One area where PGF actions differ from the activities of GH is lipid metabolism. Whereas chronic adminis-

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tration of GH leads to growth with fat mobilization and a reduction of carcass fat (21), chronic PGF treatment (plerocercoid infection) is lipogenic and stimulates an increase in carcass fat mass (11, 22). The fact that continuous exposure to PGF via plerocercoid infection in rodents did not stimulate lipolysis (11), reduce sensitivity to insulin, or alter serum insulin levels (23) suggests that PGF may not possess anti-insulin/diabetogenic actions. In this study we compared the anabolic and diabetogenic activities of GH with injections of PGF.

Materials and Methods

Preparation and Quantitation of PGF. All stages of the life cycle of *S. mansonoides* are maintained in our laboratory based on methods described by Mueller (24). For the current studies, PGF solubilized from purified plerocercoid membranes (25) was used without further purification. Previous studies involving *in vitro* metabolic assays (13, 14) and body weight gain assays (25, 26) indicate that the effective dose of PGF is not dependent on its purity (specific activity), but rather on the total activity used. Quantitation of PGF was based on a radioreceptor assay using liver membranes from late pregnant rabbits, ^{125}I-labeled hGH and hGH (NIH, hGH-I-1 2.2 IU/mg) as the standard (15). The binding activities of at least three dilutions (1/10, 1/15, 1/20) of each PGF preparation were assayed in duplicate. The quantity of PGF was expressed as the binding activity in nanogram equivalents (ng eq) of the hGH standard (25). The total activity for the PGF used in the current studies was 1328 ng eq/ml, and the specific activity was 553 ng eq/mg protein.

Animal Treatment. Hereditary obese (*ob/ob*) mice are very sensitive to the diabetogenic action of GH (1). Eight-week-old females C57BL/6J obese (*ob/ob*) mice weighing 45–55g were obtained from The Jackson Memorial Laboratory (Bar Harbor, ME). The mice were housed in groups of 5–7 animals/cage in a temperature-controlled room (25 ± 1°C) on a 12-hr light/12-hr dark cycle, and they had free access to water and Purina Rodent Chow (Ralston Purina, St. Louis, MO). The mice were weighed and handled daily for 2 weeks prior to the initiation of any experimental treatment.

To determine an effective dose range for PGF in mice, the stimulation of body growth in phenotypically normal (C57BL/6J) age-matched female mice from the same colony as the obese mice was assessed. Twenty-five mice (19–23 g) were divided into five equal groups and were injected daily subcutaneously for 10 days with phosphate-buffered saline (pH 7.4), bovine GH (100 μg/day) (NIH, GH-G-18, 1.5 IU/mg), or various concentrations of PGF (12.5, 25, and 100 ng eq/day). All injections were in the same volume (300 μl) which contained 10% polyvinylpyrrolidone to prolong the effective life of peptides (27). The mice were weighed daily.

Diabetogenicity of hGH and PGF was evaluated in obese mice as described by Reagan (1). In order that all of the mice served as their own control, they were first injected subcutaneously once a day for 3 days with 300 μl of 10% polyvinylpyrrolidone in phosphate-buffered saline. On the fourth day, the mice were injected subcutaneously with 2 μg of dexamethasone sodium phosphate (Organon Inc., West Orange, NJ), in sodium citrate buffer (pH 7.4) and fasted for 8 to 10 hr. Blood samples (25 μl) were collected by retro-ocular sinus puncture in unanesthetized mice using narrow graduated capillary tubes (Fisher Scientific, Pittsburgh, PA) and were squirted into plastic micro-test tubes (Sarsdedt, Princeton, NJ) and placed on ice. The mice were then injected intraperitoneally with 200 μl of a glucose solution (1 mg/g body wt). Blood samples were collected before and at intervals of 15, 30, 60, 90, 120, and 180 min following the injection of glucose. To all of the blood samples, 75 μl of a 5% $ZnSO_4 \cdot 7H_2O$ solution and 75 μl of a 0.3 *N* $Ba(OH)_2$ solution were added to precipitate blood protein and cells. The samples were centrifuged at 13,000*g* for 5 min and the clear supernatant was collected and used for glucose determination in triplicate by the glucose oxidase method using a Glucose Analyzer-2 (Beckman Instruments, Inc., Fullerton, CA). The obese mice were then allowed to "rest" for 7 to 10 days before the test substances were administered. After the resting period, groups of mice were injected with either 100 μg of hGH or various doses of PGF (50–200 ng eq/day) daily for 3 days. On the fourth day, the glucose tolerance test was repeated as described above. The dose of hGH used (100 μg/day) was previously shown to be very effective in producing a rise in fasting blood glucose concentration and an impairment of glucose tolerance in the obese mouse (1, 7).

Statistical Analysis. The results are expressed as mean ± SE. Statistical comparison of means of the groups was accomplished using Student's *t* test. Probability values less than 0.05 were considered to be statistically significant. Linear regression analysis was used in the determination of the correlation coefficient (*r*) of the dose-response curve. When more than two comparisons were made, the data were analyzed by one-way analysis of variance followed by Scheffe's test for multiple comparisons using the CRISP computer program (Crunch Software, Oakland, CA).

Results

Body Weight Gain in Mice. The mice treated with bGH for 10 days had a total weight gain of 4.1 ± 1.7 g which was significantly greater ($P < 0.01$) than that of the saline-treated control mice (1.4 ± 0.8 g) (Fig. 1). The mice injected with PGF responded in a dose-dependent manner ($r = 0.83$). The growth curves represented in Figure 1 show that the 100-ng eq dose of PGF produced a response which was superior to all of the other treatments. However, 25 ng eq of PGF each

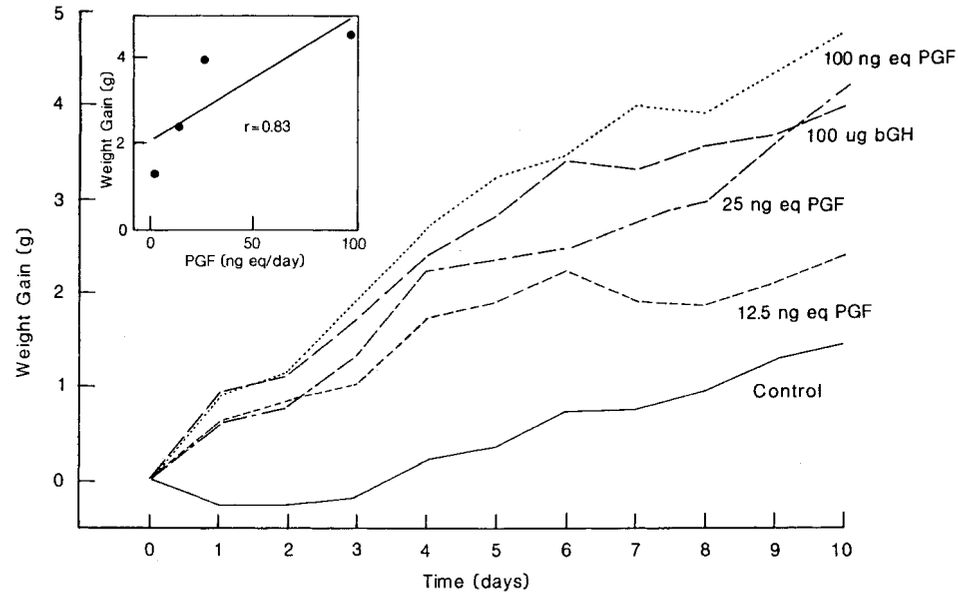

Figure 1. Growth curves of phenotypically normal C57BL/6J female mice injected with hGH or PGF. The mice were injected daily, for 10 days, with saline (control), bGH (100 μg/mouse), or various doses of PGF (12.5–100 ng eq/mouse) and body weight gain of the individuals in each group was determined. Each point represents the mean weight gain of five mice. The inset represents linear regression analysis of the doses of PGF vs body weight gain. $P < 0.01$ for PGF (25 and 100 ng eq/day) and bGH vs saline-treated control with response to total weight gain.

day stimulated a total weight gain (4.1 ± 1.8 g) which was significantly greater than that of the control group (1.4 ± 0.8 g, $P < 0.01$), but the response to 25 ng eq of PGF was similar in magnitude and not statistically different from that produced by 100 μg of bGH/day (4.1 ± 1.7 g) or 100 ng eq of PGF/day (4.5 ± 1.9 g). Therefore, the dose of PGF which gave an unequivocal response in the weight gain assay was 25 ng eq/day or 1.3 ng eq/day/g body wt. As the average weight of the obese (*ob/ob*) mice was 50.3 ± 1.3 g, an equivalent dose of PGF required to produce a similar response was calculated to be 65 ng eq/day (50.3 g × 1.3 ng eq/day/g). In order to ensure that a sufficient amount of PGF was used, doses ranging from 50 to 200 ng eq/day were used to test diabetogenicity.

Glucose Tolerance Test. Treatment with PGF stimulated body growth in normal mice, but it did not affect the fasting blood glucose levels or alter glucose tolerance of the *ob/ob* mice. Similar to the earlier reports by Reagan (1) and Kostyo *et al.* (7), treatment of obese mice with hGH for 3 days resulted in a significant increase in fasting blood glucose ($P < 0.005$), and the glucose tolerance curve was significantly impaired ($P < 0.01$) at each collection point of the 3-hr test compared with control data for the same group (Fig. 2). In contrast, treatment of the obese mice with amounts of PGF over three times that required to stimulate an unequivocal growth response in normal mice did not alter the fasting blood glucose levels, nor was glucose tolerance impaired compared with that of controls (Figs. 3 and 4). In some cases, it appeared that

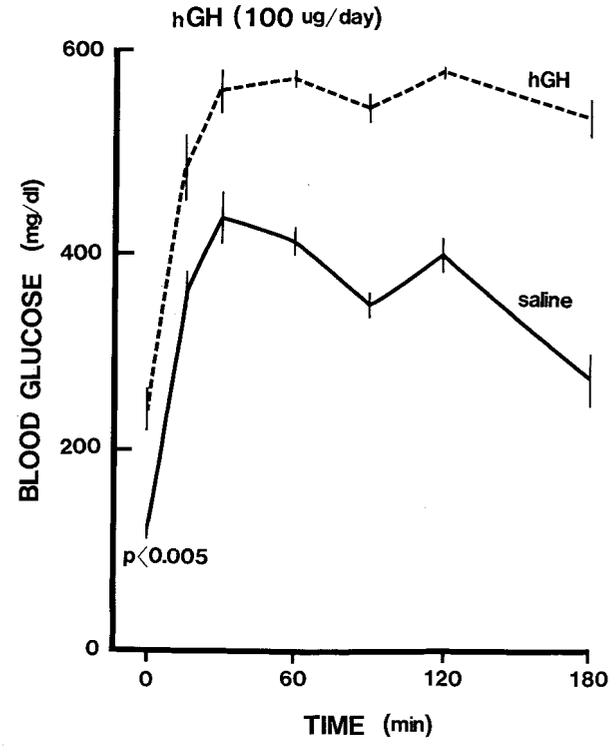

Figure 2. Effects of injections of hGH on fasting blood glucose levels and glucose tolerance of C57BL/6J-ob obese (*ob/ob*) mice. Female obese mice were injected with saline (control) or hGH (100 μg/mouse) for 3 days and a glucose tolerance test was conducted in the fasted mice as described in Materials and Methods. Each point represents the mean serum glucose concentration from five mice and the vertical lines represent ±SE. Fasted blood glucose levels of mice treated with hGH were significantly higher ($P < 0.005$) and the glucose tolerance curves were impaired at each collection point ($P < 0.01$).

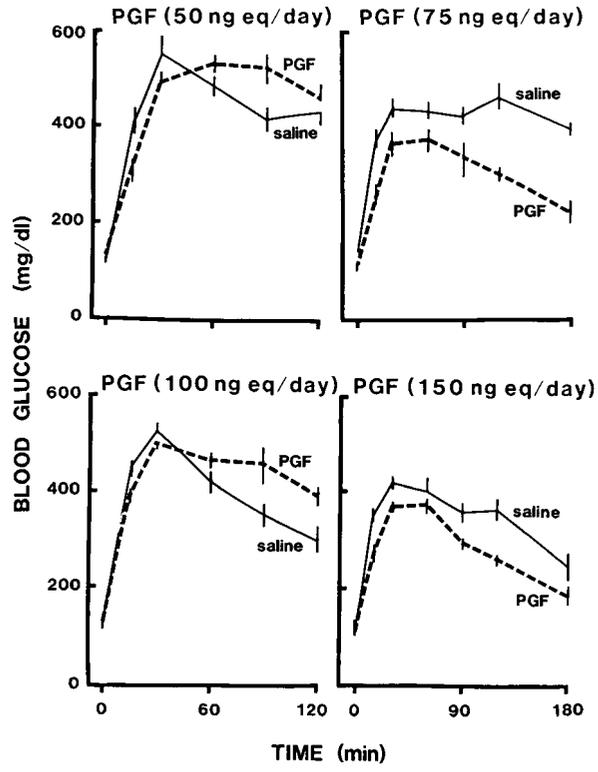

Figure 3. Effects of injections of PGF on fasting blood glucose levels and glucose tolerance of C57BL/6J-ob obese (*ob/ob*) mice. Female obese mice were injected with saline (control) or PGF (50–150 ng eq/mouse) for 3 days and glucose tolerance tests were conducted in the fasted mice as described in Materials and Methods. Each point represents the mean serum glucose concentration from five mice and the vertical lines represent ±SE. Fasting blood glucose of all PGF-treated groups were unaltered compared with the control values. Mice treated with 75 or 150 ng eq/day of PGF had glucose tolerance curves which were slightly but significantly improved compared with the controls ($P < 0.05$).

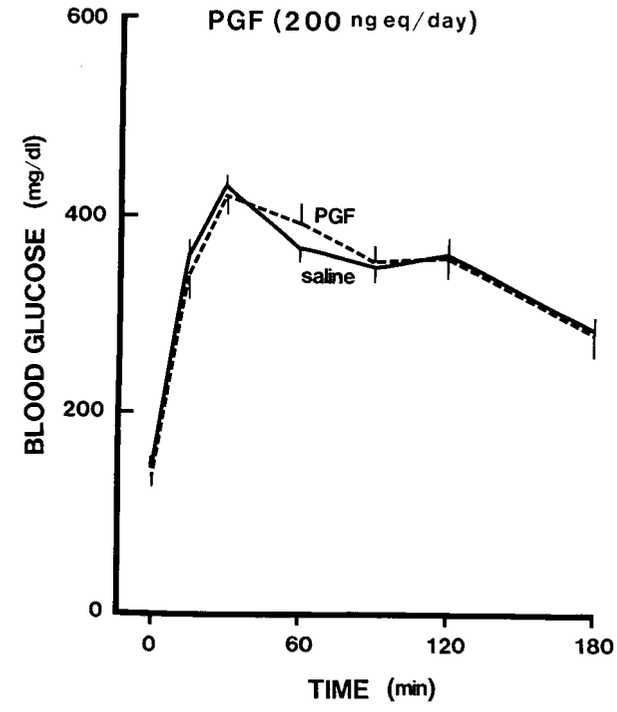

Figure 4. Effects of injections of PGF on fasting blood glucose levels and glucose tolerance of C57BL/6J-ob obese (*ob/ob*) mice. Female obese mice were injected with saline (control) or PGF (200 ng eq/mouse) for 3 days and a glucose tolerance test was conducted as described in the legend of Figure 3. Each point represents the mean serum glucose concentration from 14 mice and the vertical lines represent ±SE. Treatment with PGF did not increase fasting blood glucose or impair glucose tolerance at any point when compared with control data from the same mice.

injections of PGF may have improved glucose tolerance in obese mice. For example, Figure 3 shows that obese mice injected with 75 or 150 ng eq/day of PGF had glucose tolerances which were significantly improved compared with those of the controls ($P < 0.05$), but showed no alteration in their fasting blood glucose levels. However, as these results were not consistently observed at other PGF doses and were not observed in obese mice injected with the highest dose of PGF (200 ng eq/day) (Fig. 4), no beneficial effect of PGF can be assumed.

Discussion

This work demonstrates that PGF was a potent growth-enhancing factor in normal mice, but doses of PGF far in excess of that found to be effective in the weight gain assay did not increase fasting blood glucose, or impair glucose tolerance of obese mice as hGH did.

There is convincing evidence which suggests that the multiple activities of GH reside in distinct active sites of the GH molecule which may be expressed independently (8). It is not known if a single GH receptor can be differentially activated to initiate several distinct transmembrane signals or if there are multiple receptors for GH. Although some heterogeneity of GH receptors has been reported, there is no evidence for the existence of individual receptors for GHs anabolic, diabetogenic, and insulin-like activities. Since there have been no native GH reported which do not express anabolic, diabetogenic, and insulin-like activities and there is no precise knowledge of the location of the active sites in the GH molecule, elucidation of the basis of GH's multiple activities has been difficult.

The present study provides data that PGF has somatogenic activity but may be devoid of the diabetogenic characteristic of GH. The maximum PGF dose used (200 ng eq/day) was eight times the absolute amount required to stimulate a dramatic and unequivocal body growth response in normal mice, but was not diabetogenic. Furthermore, in support of the effective dosage used on mice, previous studies show that doses of PGF as low as 50 ng eq/day stimulated significant increases in body growth of hypophysectomized rats (25, 26) and induced acute and chronic insulin-like effects in adipose tissue and skeletal muscle of normal and hypophysectomized rats (14). Other studies using normal mice of the BALB/c strain showed that PGF treatment (plerocercoid infection) for 11 to 18 weeks

did not impair glucose tolerance or decrease glucose uptake by the tissues (23). Sensitivity of adipose tissue and skeletal muscle to insulin *in vitro* was increased in the chronically PGF-treated rats (12), and injections of partially purified PGF produced acute insulin-like effects in tissues from normal rats (14). Furthermore, insulin-like actions were elicited *in vitro* by adding PGF to freshly excised tissues from normal rats, indicating that refractoriness to the insulin-like actions of GH observed in tissues from normal rats had no influence on the actions of PGF (13).

Since the PGF used in this study was not homogenous, it is possible that some component of the partially purified preparations could have somehow differentially interfered with the expression of diabetogenicity by PGF. This seems unlikely as it is clear from our current results and earlier reports that regardless of whether PGF is administered via plerocercoid infection (11, 12, 22) or by injection of partially purified material (14, 25, 26), both growth-stimulating and insulin-like activities are expressed. In any event, our current work shows that it is possible for a GH-like factor to stimulate growth but not elicit diabetogenic activity.

Cross-reactivity of PGF with specific anti-hGH monoclonal antibodies (19) and the ability to displace hGH from its receptors (15–18) and to duplicate many biological actions suggest remarkable similarity between PGF and hGH. However, marginal cross-reactivity with polyclonal anti-hGH antibodies (19) and reduced binding affinity in some cell types (13, 18) suggest that PGF and hGH are not identical. Therefore, PGF may closely resemble hGH in the molecular region(s) responsible for the stimulation of growth and insulin-like actions, but some molecular difference renders PGF incapable of stimulating diabetogenic/anti-insulin activities, at least in the dose range used in this study. Therefore, our data support the hypothesis that there are distinct "active cores" in the hGH molecule and suggest that the growth-promoting and diabetogenic activities of hGH are independent of each other.

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