In Vitro Insulin-Like Actions of the Growth Factor from the Tapeworm, *Spirometra mansonoides* (42851)

MOHAMMED A. M. SALEM[1] AND C. KIRK PHARES
Department of Biochemistry, University of Nebraska Medical Center, Omaha, Nebraska 68105

Abstract. In vitro **actions of purified plerocercoid growth factor (PGF) were compared with those of insulin and human growth hormone (hGH) in adipose tissue from normal male rats. Insulin-like effects were measured by the ability of PGF, insulin, or hGH to stimulate oxidation of [U-^{14}C]glucose to $^{14}CO_2$, to stimulate lipogenesis, and to inhibit epinephrine-induced lipolysis. PGF and insulin stimulated significant increases in glucose oxidation and lipogenesis in adipose tissue that had not been preincubated as well as in tissue that had been preincubated. hGH stimulated insulin-like effects only in tissue that had been preincubated for 3 hr. Insulin, hGH, and PGF inhibited epinephrine-induced lipolysis of preincubated (3 hr) adipose tissue. hGH produced a dramatic lipolytic response in tissue freshly removed from normal rats but no dose of PGF was lipolytic. PGF did not displace ^{125}I-insulin from its receptors on adipocytes but did competively inhibit ^{125}I-hGH binding to adipocytes. These results suggest that PGF has direct insulin-like actions which are initiated by binding a GH receptor, but PGF had no anti-insulin action and the insulin-like activity of PGF was unaffected by refractoriness of adipose tissue to GH.** [P.S.E.B.M. 1989, Vol 190]

A factor produced by plerocercoids of the tapeworm *Spirometra mansonoides* is structurally and functionally similar to human growth hormone (hGH). Like hGH, plerocercoid growth factor (PGF) stimulates body growth in rodents (1–3), increases somatomedin production (4, 5), binds to somatogenic and lactogenic receptors (6–8), cross-reacts with anti-hGH antibodies (9), and is lactogenic in the pigeon crop-sac assay (10). Although the biologic similarities between hGH and PGF are many there are distinct differences. Growth hormone is lipolytic (11) and diabetogenic (12) whereas PGF is distinctly lipogenic (13–16) and is not diabetogenic (3).

It is well established that growth hormones (GH) from a variety of species exhibit multiple biologic activities. In addition to the stimulation of growth, GH has intrinsic anti-insulin properties as well. Furthermore, under special circumstances, GH produces transient insulin-like effects, including stimulation of glucose uptake and utilization as well as the inhibition of lipolysis. Normal animals or tissues from normal animals are sensitive to the anti-insulin actions of GH, but the insulin-like actions of GH are best demonstrated in tissues from GH-deficient animals such as very young fasted rats (18), hypophysectomized rats (19), or normal rats treated with anti-GH antibodies (20). The refractoriness (insensitivity) of tissues from normal animals to the insulin-like actions of GH is believed to be due to some action of GH itself (21). The mechanism responsible for refractoriness is not understood, but several possibilities have been proposed. The suggestion that reversal of refractoriness requires *de novo* protein synthesis (19) is not totally satisfactory in light of evidence which demonstrates that tissues removed from stressed normal rats respond immediately to the insulin-like action of GH (20). Furthermore, refractoriness is not due to an inadequate number of GH receptors or to decreased affinity of GH binding (23) as hypophysectomized rats have fewer GH receptors than normal rats, yet are very sensitive to the insulin-like actions of GH (24, 25). Since tissues which are refractory to the insulin-like action of GH are fully responsive to insulin and do not become refractory to insulin after repeated exposure, there is obviously no defect in the tissue's ability to respond to an appropriate signal. Eden *et al.*

[1] Present address: The Whittier Institute for Diabetes and Endocrinology, Scripps Memorial Hospital, 9894 Genesee Avenue, La Jolla, CA 92037.

Received January 27, 1988. [P.S.E.B.M. 1989, Vol 190]
Accepted October 27, 1988.

0037-9727/89/1902-0203$2.00/0

(26) suggested that in tissues refractory to the insulin-like action of GH, the GH receptor may not be coupled to the appropriate effector mechanism in the mem brane. If this is true, GH would bind its receptor but no transmembrane signal would be transmitted to initiate the insulin-like responses.

Although the GH-like factor from plerocercoids of *S. mansonoides* stimulates growth in a variety of species (1), the type of growth stimulated by PGF is somewhat different from that produced by mammalian GH. Growth hormone stimulates lean body growth whereas PGF stimulates growth with fattening (1, 13, 14). This suggests an insulin-like action for PGF and is supported by our recent observation that injections of PGF into normal rats stimulated acute insulin-like effects in adipose tissue (27). The purpose of the current work was to compare the direct *in vitro* effects of PGF with those of hGH and insulin on receptor binding and metabolic activity of adipose tissue from normal male rats.

Materials and Methods

Preparation of PGF. Plerocercoids were removed from mice, homogenized, and a membrane fraction was separated and solubilized in the nonionic detergent Triton X-100 (Sigma Chemical Co., St. Louis, MO) as described previously (2). In some cases the solubilized PGF was used directly as the test material but in other instances the PGF was subjected to further purification by either chromatofocusing chromatography (28) or hGH receptor affinity chromatography (2). Quantitation of both crude and purified PGF was based on its ability to competitively displace ^{125}I-hGH from receptors in a rabbit liver membrane radioreceptor assay (RRA). The RRA was conducted as originally described (6) except an overnight (16–18 hr) incubation was used and Triton X-100 (0.1% v/v) was included in the sample diluting buffer. Concentrations of PGF were expressed as nanogram equivalents (ng eq) of the hGH standard. Three different preparations of PGF were used in these studies and the total activities and specific activities were as follows: crude PGF, 308 ng eq/ml and 42 ng eq/mg protein; PGF purified by chromatofocusing, 1328 ng eq/ml and 553 ng eq/mg protein; receptor affinity purified PGF, 1512 ng eq/ml and 12,400 ng eq/mg protein. No differences in tissue response to equivalent doses of PGF from any of the three different preparations were observed. Therefore, only the doses (in ng eq/ml) of PGF used in the various experiments were denoted.

Prior to the use of crude PGF for further purification or for the *in vitro* metabolic studies, most of the Triton X-100 was removed by batch treatment with SM-2 Biobeads (BioRad Labs, Richmond CA). More than 99% of a 1% solution (v/v) of Triton X-100 can be removed by this method (29). In preliminary studies we found that very low concentrations (<0.03%) of Triton X-100 inhibited glucose metabolism in rat adipose tissue by as much as 90%. To overcome this problem, Triton X-100 was further extracted from all preparations of PGF used in the current studies by a modification of the method of Horigome and Sugano (30). Bovine serum albumin (BSA) (3 mg/ml) was dissolved in each preparation of PGF prior to the addition of Fractogel HW-40F (EM Reagents, Gibbstown, NJ) in a ration of 1 ml of gel slurry to 3 ml of PGF. The slurry was gently mixed at 4°C for 30 min and the PGF-BSA solution was separated from the gel by filtration over a glass-wool filter. The preparations of PGF were used within 4 hr following the second detergent extraction. Greater than 90% of the activity of the PGF preparations (as determined by RRA) was recovered. A control sample containing 1% Triton X-100 and BSA prepared and extracted in the same manner as the PGF samples had no effect on glucose metabolism in adipose tissue.

Animals and Adipose Tissue Preparation. Young normal male Sprague-Dawley rats (140–180 g) were obtained from SASCO (St. Louis, MO) and were fed *ad libitum* with standard Purina Lab Chow (Ralston Purina, St. Louis, MO). The rats were killed by decapitation between 0900 and 1100 hr on the day of the experiments. The thin distal portions of the epididymal adipose tissue were quickly removed, placed in Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4 (previously gassed thoroughly with 95% O_2-5% CO_2), cut into small segments (30–50 mg each), and randomly aliquoted into various assay flasks (100–150 mg of tissue segments/flask) as described previously (21).

Glucose Metabolism. It has been reported that endogenous GH induces a condition of refractoriness in adipose tissue to its own insulin-like actions resulting in the absence of effects on this tissue obtained from normal animals when assayed within 1 hr after removal (21, 31). To compare the actions of PGF to those of hGH and insulin on tissue which should be insensitive to the insulin-like actions of GH, some tissue aliquots were transferred directly into KRB buffer (pH 7.4) containing 5.5 m*M* glucose ([U-^{14}C]glucose, 0.027 μCi/μ*M*/ml added as tracer) with or without hormone (1 μg/ml of hGH or 100 microunits/ml of insulin) or various concentrations of PGF (5–100 ng eq/ml) and incubated for 1 hr at 37°C. The hGH (NIH, hGH I-1, 2.2 IU/mg) was provided by the National Hormone and Pituitary Agency, NIDDK. The insulin used was regular Iletin II (Eli Lilly and Co., Indianapolis, IN).

The insensitivity of adipose tissue from normal rats to the insulin-like actions of GH can be relieved if the tissues are preincubated for 2 to 3 hr in the absence of GH (21). Therefore, other aliquots of adipose tissue were transferred to KRB buffer without hormones, PGF, or [U-^{14}C]glucose and were preincubated for 3 hr prior to transfer to fresh KRB buffer containing 5.5

m*M* glucose with [U-^{14}C]glucose added as the tracer in the presence of hormones or PGF and incubated for an additional 1 hr at 37°C. The reactions were terminated by adding 0.5 ml of 1 *N* H_2SO_4. The $^{14}CO_2$ liberated was trapped in tightly coiled strips of filter paper in suspended polyethylene collection cups (Kontes, Evanston, IL) containing hyamine hydroxide 10-X (Sigma, St. Louis, MO) as described previously (22). To measure the amount of lipid synthesized, the incubated adipose tissue was homogenized and extracted for total ^{14}C lipids using Dole's extraction mixture (heptane:isopropanol:1 *N* H_2SO_4; 40:10:1) (32). Radioactivity was counted in a Tri-Carb 460 liquid scintillation spectrometer (Packard Instruments, Downers Grove, IL).

Antilipolytic Effect. To measure antilipolytic effects, tissue segments were preincubated in KRB buffer (containing 1% BSA, 1 mg/ml glucose) for 3 hr as described above. Following the preincubation period, the tissue segments were transfered to fresh control medium (KRB buffer, 4% BSA, 1 mg/ml glucose) or to medium containing epinephrine (50 ng/ml) alone (to stimulate lipolysis), or to medium containing a combination of epinephrine with either PGF (5–150 ng eq/ml), hGH (1 μg/ml), or insulin (100 microunits/ml). All incubations contained 0.1 mg/ml ascorbate to prevent the oxidation of epinephrine. The incubations were continued for 1 hr at 37°C in a shaking water bath. The reaction was terminated by quickly placing the assay flasks on ice and removing the tissue segments from the flasks with forceps. Glycerol released into the medium during incubation was used as an index of lipolysis. The assay medium was diluted 1/3 with KRB buffer and glycerol analysis was performed using an enzymatic flourometric method originally described by Wieland (33) and modified by Nilsson and Belfrage (34). The advantage of using glycerol over free fatty acids is because of its stability and it cannot readily be metabolized by the adipose tissue and therefore gives a more reliable indication of lipolytic activity (35).

Lipolytic Effect. Whereas the *in vitro* insulin-like effects of GH can only be observed in tissues from GH-deficient rats or in tissues from normal rats after preincubation for 2 to 3 hr to remove refractoriness, anti-insulin effects of GH, including lipolysis, can be measured in tissues from normal rats without preincubation (11, 23, 36). Adipose tissue was removed and incubated for 3 hr in the absence or presence of various concentrations of hGH (10–1000 ng/ml) or PGF (10–100 ng eq/ml) in KRB buffer containing 4% BSA and 1 mg/ml glucose. The adipose tissue was then transferred to a fresh hormone-free medium containing 0.3 ng/ml theophylline for an additional hour at 37°C. Glycerol released into the medium was used as an index of lipolysis.

Binding Studies. Adipocytes were isolated from epididymal fat pads of normal rats using the method of Rodbell (37) with some modifications. The fat pads were cut into small pieces and transferred to plastic beakers in KRB buffer containing 4% BSA, 1 mg/ml glucose, 1 mg/ml collagenase (Cooper Biomedical Diagnostics, Freehold, NJ). The beakers were thoroughly gassed with 95% O_2-5% CO_2 and sealed with Parafilm (American Can, Greenwich, CT) and incubated for 30 min in a shaking water bath at 37°C. The cells were filtered through four layers of buffer-wetted cotton gauze. Visible clumps of cells were discarded and the adipocytes were collected after centrifugation for 1 min at 400*g* at room temperature. The cake-like layer of cells was washed three to four times with KRB buffer containing 1% BSA and 1 mg/ml glucose, then resuspended in KRB buffer and the concentration of adipocytes was determined with a hemocytometer.

To determine if PGF competitively inhibits insulin binding to adipocytes, 3×10^5 cells/ml were incubated with 1×10^5 cpm of porcine ^{125}I-insulin (37 μCi/μg; New England Nuclear, Boston, MA) with increasing concentrations of insulin (2–100 ng/ml) or PGF (2–100 ng eq/ml) for 1 hr at 37°C. Separation of bound hormone from free hormone was achieved by centrifugation of adipocytes over dinonyl phthalate (38) in plastic microtest tubes (Sarsdedt, Princetown, NJ) followed by cutting the tube below the upper phase containing the cell layer with a hot razor blade. Nonspecific binding (NSB) was defined as the amount of ^{125}I-insulin bound in the presence of a large excess of unlabeled insulin (25 μg/ml). Total binding was defined as the amount of labeled hormone bound in the absence of unlabeled hormone. Specific binding was obtained by subtracting the NSB from total binding and was expressed as a percentage of the total radioactivity added to each tube.

Competitive binding studies using adipocytes, ^{125}I-hGH (6×10^4 cpm, 60 μCi/μg), and hGH or PGF were also conducted. The procedures and the calculations of ^{125}I-hGH binding were the same as those described for the insulin binding studies.

Statistical Analysis of Data. All data are presented as the mean ± SE. The dose-response curves were analyzed by logarithmic regression and the competitive displacement curves for the binding studies were analyzed by logit-log regression. The data for the various treatment groups were subjected to Student's *t* test and *P* values less than 0.05 were considered statistically significant.

Results

Glucose Metabolism. Consistent with previous reports (21, 22) adipose tissue removed from normal rats was resistant to the insulin-like actions of hGH added to the incubated tissue within the first hour after removal. Preincubation for 3 hr removed the refracto-

riness of the adipose tissue to the insulin-like action of hGH (Fig. 1). Remarkably, PGF stimulated significant increases in glucose oxidation when added to adipose tissue directly after removal from the rats as well as when added after preincubation (Fig. 1). The potency of PGF in stimulating glucose metabolism is noteworthy as 32 ng eq/ml stimulated a response which was not significantly different from that produced by 1 μg/ml of hGH in tissues that had been preincubated for 3 hr prior to the addition of hormone. Furthermore, the data in Figure 2 show that PGF stimulated the rate of glucose oxidation as well as the incorporation of ^{14}C from glucose into total lipids of adipose tissue segments in the first hour of incubation in a dose-dependent manner. A dose of as little as 25 ng eq/ml stimulated a significant ($P < 0.05$) increase in glucose oxidation and ^{14}C incorporation into lipids. These results clearly demonstrate that PGF has distinct insulin-like activities when added directly to adipose tissue *in vitro* and that refractoriness to GH did not affect the insulin-like actions of PGF. This supports our earlier observation that injections in PGF in normal rats produced acute increases in glucose and leucine metabolism in adipose tissue and skeletal muscle (27).

Inhibition of Epinephrine-Induced Lipolysis. Among the direct insulin-like actions of GH is its ability to inhibit epinephrine-stimulated lipolysis in adipose tissue. However, as in the case of glucose oxidation, 2–

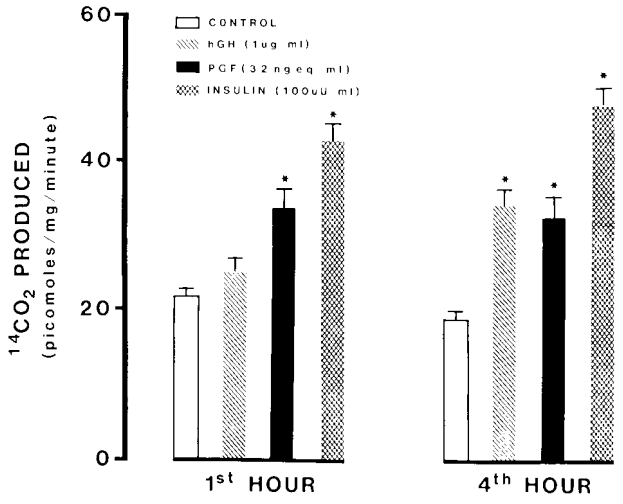

Figure 1. Effect of hGH, insulin, or PGF on glucose oxidation in adipose tissue from normal rats. Adipose tissue was removed from normal male rats, cut into small segments, which were either placed directly in assay medium containing [U-^{14}C]glucose with hGH (1 μg/ml), insulin (100 microunits/ml), or PGF (32 ng eq/ml) for 1 hr (first hour), or preincubated in the absence of hormones or PGF for 3 hr before being transferred to the assay medium containing [U-^{14}C] glucose and hormones in the final hour of incubation (fourth hour). Oxidation of [U-^{14}C]glucose to $^{14}CO_2$ in adipose tissue was measured as described in Materials and Methods. The data are expressed as mean ± SE of quadruplicate samples and are the results of three separate experiments. Asterisks indicate statistically significant differences in the rate of glucose oxidation vs the control ($P < 0.05$).

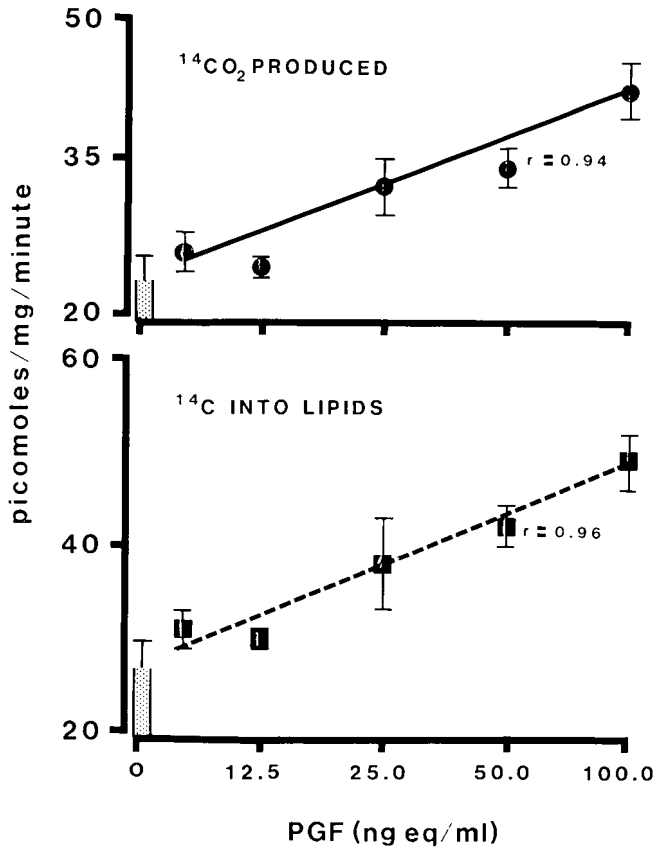

Figure 2. Effect of increasing doses on stimulation of glucose metabolism in adipose tissue from normal rats. Adipose tissue was removed from normal male rats, cut into small segments, and placed directly into assay medium containing [U-^{14}C]glucose in the presence of buffer alone (control) or PGF (5–100 ng eq/ml). The rates of [U-^{14}C]glucose oxidation to $^{14}CO_2$ and ^{14}C incorporation into lipids in adipose tissue were measured as described in Materials and Methods. The data are expressed as mean ± SE of quadruplicate samples and are the results of two separate experiments. Upper panel, [U-^{14}C]glucose oxidation to $^{14}CO_2$ in adipose tissue. Lower panel, ^{14}C incorporation into lipids of adipose tissue. Addition of PGF produced a highly significant ($P < 0.05$) dose-dependent increase in glucose oxidation and ^{14}C incorporation into lipid of adipose tissue. At concentrations of 25 ng eq/ml and above, PGF produced significant increases over the control ($P < 0.05$).

3 hr of preincubation are required to allow tissues to escape from refractoriness to this action of GH (39, 40). In order to ensure sensitivity to GH, we preincubated adipose tissue segments for 3 hr prior to the addition of hormones or PGF. The data in Figure 3 show that insulin (100 microunits/ml) inhibited epinephrine-stimulated lipolysis by 33% ($P < 0.05$) and hGH (1 μg/ml) inhibited lipolysis by 29% ($P < 0.05$) when compared with control tissue to which only epinephrine was added. Addition of increasing concentrations of PGF also resulted in a dramatic inhibition of lipolysis and 10 ng eq/ml of PGF caused a level of inhibition (24%) which was significantly ($P < 0.05$) different from the control and of similar magnitude as that produced by 1 μg/ml of hGH. At 100 ng eq/ml of PGF, the magnitude of the inhibition was not significantly differ-

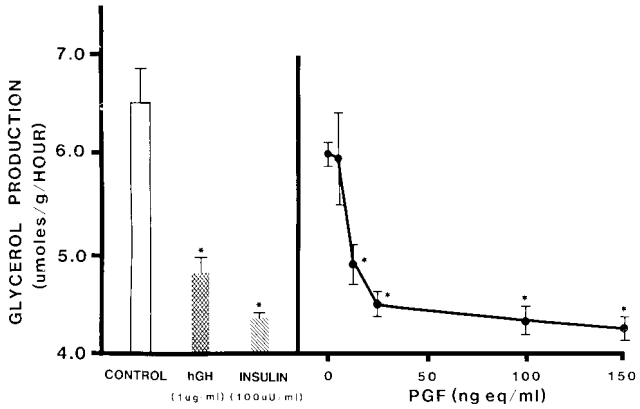

Figure 3. Effect of hGH, insulin, and PGF on epinephrine-induced lipolysis in adipose tissue from normal rats. Adipose tissue segments were removed from normal male rats and incubated for 3 hr in buffer alone. The tissues were then transferred to a fresh medium containing 50 ng/ml of epinephrine alone (control), epinephrine plus insulin (100 microunits/ml), epinephrine plus hGH (1 μg/ml), or epinephrine plus PGF (5–150 ng eq/ml) for 1 hr. The amount of glycerol released into the medium in the final hour of incubation was measured as described in Materials and Methods. The data are the mean ± SE of quadruplicate samples and are the results of a single representative experiment from three separate experiments. Asterisks indicate statistically significant differences in glycerol production vs the control ($P < 0.05$).

ent from that produced by 100 microunits/ml of insulin. Therefore, the presence of antilipolytic activity confirms that PGF has intrinsic insulin-like activities.

Stimulation of Lipolysis. Preincubation is not necessary to demonstrate anti-insulin activities of GH as tissues from normal as well as hypophysectomized rats are sensitive to the lipolytic action of GH when it is added directly to tissue (11). The data in Figure 4 show that the lowest concentration of hGH used (10 ng/ml) stimulated a significant increase in lipolysis and that the response was dose related ($r = 0.95$) through the highest dose of hGH (1000 ng/ml). The highest dose of PGF (100 ng eq/ml) did not produce an increase in lipolysis when compared with the control tissue. These data suggest that the direct actions of PGF do not include lipolytic activity.

Binding Studies. The ability of PGF to competitively inhibit ^{125}I-insulin and ^{125}I-hGH binding to adipocytes from normal rats was also tested. In the current experiments specific binding (total binding-NSB) for ^{125}I-insulin ranged from 3 to 4% of the total radioactivity added to each tube and specific binding for ^{125}I-hGH was 2 to 3% of the total radioactivity added. Nonspecific binding averaged 25% of total binding for ^{125}I- insulin and 30% of total binding for ^{125}I-hGH. The data in Figure 5 show no concentration of PGF inhibited the binding of ^{125}I-insulin to its receptors on rat adipocytes. The fact that PGF binds to GH receptors in rats (7) is verified by its ability to competively inhibit specific binding of ^{125}I-hGH to its receptors on rat adipocytes (Fig. 6). It is clear from the data that PGF was less potent in displacing ^{125}I-hGH from rat adipocytes than it was in the rabbit liver membrane RRA. However, these results suggest that the insulin-like activities of PGF, like those of hGH, are initiated by binding to somatotrophic receptors rather than to insulin receptors.

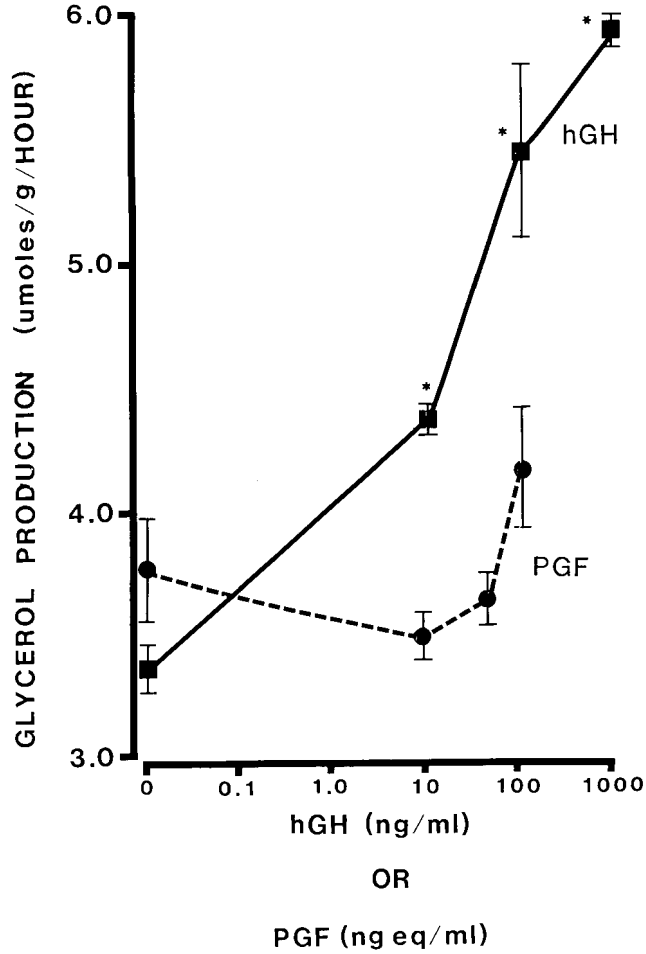

Figure 4. Effect of hGH and PGF on lipolysis in adipose tissue from normal rats. Adipose tissue was removed from normal male rats and placed directly into medium containing buffer alone (control), hGH (10–1000 μg/ml), or PGF (10–100 ng eq/ml) for 3 hr. The tissues were then transferred to fresh medium containing theophylline alone and incubated for 1 hr. The amount of glycerol released into the medium in the final hour of incubation was measured as described in Materials and Methods. The data are the mean ± SE of quadruplicate samples and are the results of a single representative experiment from three separate experiments. Addition of hGH *in vitro* produced a dose-dependent increase in lipolysis with $r = 0.95$. Asterisks indicate statistically significant ($P < 0.005$) differences vs basal glycerol production (control).

Discussion

These studies provide evidence that preparations of PGF possess intrinsic insulin-like activity when added directly to adipose tissue from normal rats. The fact that tissues from normal rats were sensitive to PGF but were refractory to hGH during the first hour of incubation suggests the possibility that PGF might act via the insulin receptors. Contrary to this idea, PGF

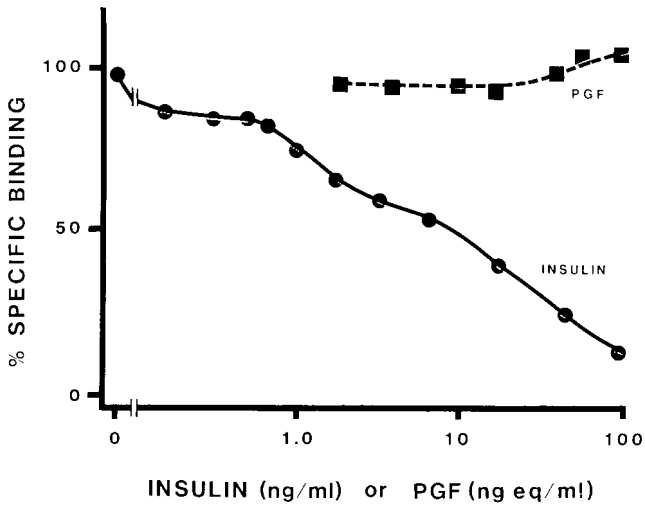

Figure 5. Competitive inhibition of ^{125}I-insulin binding to adipocytes from normal rats. Adipocytes (3×10^5 cells/ml) were prepared from normal male rats and incubated for 90 min in a medium containing ^{125}I-insulin alone or ^{125}I-insulin with either unlabeled insulin (0.2 to 100 ng/ml) or PGF (2 to 100 ng eq/ml). Binding assays were performed as described in Materials and Methods. The ordinate represents the ^{125}I-insulin specifically bound (after correcting for NSB) to the adipocytes. In this experiment, specific binding was 2.3% and NSB 0.6% of the total radioactivity added to each tube. The doses of PGF on the abscissa were based on binding activity determined in a previous RRA as described in Materials and Methods. The data shown represent the mean of duplicate samples from a single assay but are representative of three separate experiments. No dose of PGF used displaced ^{125}I-insulin from its receptors on adipocytes.

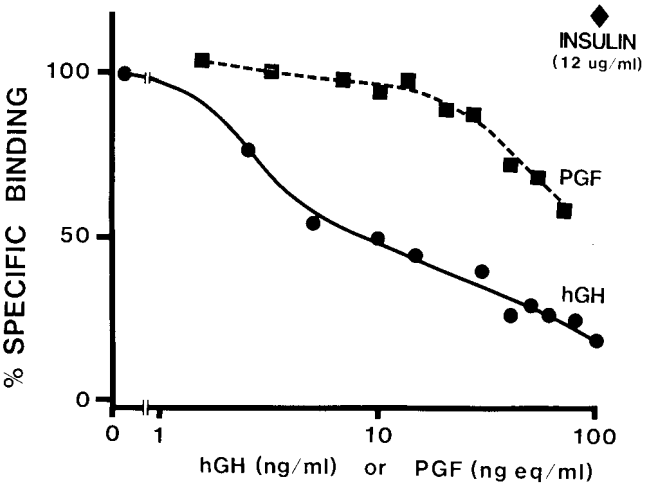

Figure 6. Competitive inhibition of ^{125}I-hGH binding to adipocytes from normal rats. Adipocytes (3×10^5 cells/ml) were prepared from normal male rats and incubated for 90 min in a medium containing ^{125}I-hGH alone or in the presence of either unlabeled hGH (0.6 to 100 ng/ml), PGF (2.5 to 67 ng eq/ml), or a single dose of insulin (12,000 ng/ml). Binding assays were performed as described in Materials and Methods. In this experiment, specific binding was 1.7% and NSB was 0.8% of the total radioactivity added to each tube. The doses of PGF on the abscissa were based on binding activity determined in a previous RRA, as described in Materials and Methods. The data shown represent the mean of duplicate samples from a single assay but are representative of four separate experiments. Addition of PGF displaced ^{125}I-hGH binding to hGH receptors on adipocytes in a dose-dependent manner with $r = 0.96$. Insulin did not competitively displace ^{125}I-hGH binding to adipocytes.

did not compete for insulin receptors but did competitively inhibit binding of ^{125}I-hGH to its receptors on adipocytes. These data are interpreted to mean that PGF induces direct effects on adipose tissue via a GH receptor. However, the responses of adipose tissue to PGF and hGH were distinctly different.

It is well known that freshly isolated tissue from normal rats is insensitive to the insulin-like effects of GH. The underlying mechanisms responsible for this lack of sensitivity is thought to be a result of the influence of endogenous GH. The possibility that refractoriness is due to a lack of GH receptors seems unlikely to be the only explanation as several groups have shown significant levels of specific binding to GH to adipocytes that were unresponsive to its insulin-like activities (23, 26). Other evidence suggests that subsequent to receptor binding and activation of a transmembrane signal, the antilipolytic effect shared by insulin and GH is the result of initiation of common metabolic pathways in adipose tissue (42). Since the same tissue which is insensitive to the insulin-like effects of GH is fully responsive to insulin seems to suggest that refractoriness to GH may be due to the GH receptors not being coupled to the appropriate effector mechanism in the plasma membrane (26).

Results of the current work clearly show that PGF specifically bound GH receptors and produced distinct insulin-like effects in adipose tissue from normal rats. Whereas we have no data regarding the differences between hGH and PGF which allowed PGF to circumvent the refractoriness of normal adipose tissue to the insulin-like effects of GH, it is clear that tissues from normal rats which had not been preincubated were highly sensitive to PGF in the same manner as observed with insulin. This suggests that refractoriness may be due to some aspect of the interaction of GH with its receptor and not the result of deficient receptors or any defect in the membrane effector system for GH as previously suggested (26). Recently, modified forms of hGH were shown to interact differently with receptors and to produce different responses in rat adipose tissue (43). These data support the possibility that the differential effects of GH on adipose tissue could be mediated by different receptors or by separate signaling mechanisms that interact with different components of the GH-receptor complex. Evidence for the presence of a functional membrane receptor effector system for GH in refractory adipose tissue is demonstrated by reports which show that when normal rats were subjected to stress, GH could stimulate insulin-like responses in adipose tissue which had not been preincubated (21, 22). Considering the apparent difference in potency of PGF in binding the GH receptor on adipocytes and initiating insulin-like responses in adipose tissue, the current data could be explained if PGF binds a putative "insulin-like" GH receptor but is unable to bind a putative "anti-insulin" GH receptor.

It is clear from the current work, as well as previous reports, that PGF can mimic some, but not all, of the actions of GH (1–10, 13–17, 44). The most obvious difference is found in the inability of PGF to duplicate the anti-insulin actions of GH (3, 13). It is possible that a distinct peptide domain of GH is responsible for its anti-insulin activities and is also required for GH to induce refractoriness to its own insulin-like activities. If PGF does not share the putative anti-insulin sequence it cannot induce refractoriness and tissues are immediately sensitive to its insulin-like actions.

The potency of PGF in producing insulin-like responses in these studies warrants attention. The quantitation of PGF for these studies was determined based on its ability to displace ^{125}I-hGH in a RRA and is expressed in ng eq of the hGH standard. The actual mass and its activity in other assays may be much greater (or less) than its ability to displace ^{125}I-hGH from its receptors in rabbit liver. In support of this is our earlier report that showed that 50 ng eq/day induced a significant weight gain response in hypophysectomized rats and 400 ng eq/day stimulated a maximal growth response (2). From the current data, it is important to note the dose-dependent nature of the insulin-like responses to PGF and that doses of PGF as low as 25 ng eq/ml significantly increased glucose metabolism and 10 ng eq/ml significantly inhibited epinephrine-induced lipolysis, yet doses of PGF up to 10 times this amount did not stimulate lipolysis when compared with control. The opposite of this relationship has been observed with GH. In order to produce a maximal insulin-like response in sensitive adipose tissue 100 to 300 ng/ml of GH is required (21, 23), but the lipolytic action of GH is observed at concentrations as low as 1 ng/ml and maximal responses are attained at concentrations of 3 to 30 ng/ml of GH (10, 24). Furthermore, refractoriness to the insulin-like actions of GH added *in vitro* is conferred by preincubating tissues with GH at concentrations of 3 ng/ml, with total refractoriness achieved at 30 ng/ml of GH (21, 23). In light of these facts, it seems reasonable to suggest that PGF is devoid of lipolytic activity. In support of this hypothesis are previous reports which show that chronic exposure to PGF results in accelerated growth with dramatic increases in fat stores of normal (1, 14) and hypophysectomized animals (13) but does not increase the level of plasma free fatty acids in hypophysectomized rats (13).

PGF specifically displaced hGH from receptors on adipocytes from normal rats and adipose tissue segments which were refractory to the insulin-like actions of hGH showed no such insensitivity to PGF. These results suggest that there is no defect in the membrane signaling system for GH in normal adipose tissue but that refractoriness may be caused by some aspect of the GH-receptor interaction that PGF is not able to duplicate.

This work was supported by NIH Grant 17226. We thank Brenda Schomber for her assistance in preparing the manuscript.

1. Mueller JF. The biology of *Spirometra*. J Parasitol **60**:3–14, 1974.
2. Phares CK. Use of receptor affinity chromatography in purification of the growth hormone-like factor produced by plerocercoids of the tapeworm, *Spirometra mansonoides*. J Recept Res **8**:645–665, 1988.
3. Phares CK, Salem MAM. The human growth hormone analogue produced by the tapeworm, *Spirometra mansonoides*, is not diabetogenic in the obese (ob/ob) mouse. 68th Annual Meeting of the Endocrine Society, p218, 1986.
4. Garland JT, Ruegamer WR, Daughaday WH. Induction of sulfation factor activity by infection of hypophysectomized rats with *Spirometra mansonoides*. Endocrinology **88**:924–927, 1971.
5. Phares CK, Ruegamer WR. Effects of the growth factor from the tapeworm, *Spirometra mansonoides*, on a hypophysectomized rhesus monkey. 58th Annual Meeting of the Endocrine Society, p286, 1976.
6. Tsushima T, Friesen HG, Chang TW, Raben MS. Identification of sparganum growth factor by a radioreceptor assay for growth hormone. Biochim Biophys Res Commun **59**:1062–1068, 1974.
7. Phares CK, Booth BJM. Reduction of lactogenic receptors in female hamster liver due to the human growth hormone analog produced by plerocercoids of the tapeworm, *Spirometra mansonoides*. Endocrinology **118**:1102–1109, 1986.
8. Phares CK. Suppression of receptors for human growth hormone and estradiol in female rats treated with the growth hormone analogue produced by plerocercoids of the tapeworm, *Spirometra mansonoides*. J Recept Res **6**:425–431, 1986.
9. Phares CK, Booth BJM. Anti-human growth hormone antibodies crossreact with the growth hormone-like factor from plerocercoids of the tapeworm, *Spirometra mansonoides*. Endocrinology **121**:1839–1844, 1987.
10. Phares CK, Ruegamer WR. Pigeon crop-sac stimulation due to a factor produced by plerocercoids of the tapeworm, *Spirometra mansonoides*. Proc Soc Exp Biol Med **143**:147–151, 1973.
11. Goodman HM, Grichting G. Growth hormone and lipolysis: A reevaluation. Endocrinology **113**:1697–1702, 1983.
12. Reagan CR. The diabetogenic activity of fragments of human growth hormone in obese (ob/ob) mice. Diabetes **27**:883–888, 1978.
13. Steelman SL, Glitzer MS, Ostlind DA, Mueller JF. Biological properties of the growth hormone-like factor from the plerocercoid of *Spirometra mansonoides*. Recent Prog Horm Res **27**:97–120, 1971.
14. Phares CK, Carroll RM. A lipogenic effect in intact male hamsters infected with plerocercoids of the tapeworm, *Spirometra mansonoides*. J Parasitol **63**:690–693, 1977.
15. Phares CK, Carroll RM. Comparison of the effects of the growth factor produced by *Spirometra mansonoides* and growth hormone in diabetic-hypophysectomized rats: Lipid composition. J Parasitol **64**:401–405, 1978.
16. Phares CK, Carroll RM. Insulin-like effects on fatty acid synthesis in liver of hamsters infected with plerocercoids of the tapeworm, *Spirometra mansonoides*. J Helminthol **58**:25–30, 1984.
17. Sadun EH, Williams JS, Maroney FC, Mueller FC. Biochemical changes in mice infected with spargana of the cestode, *Spirometra mansonoides*. J Parasitol **51**:532–536, 1965.
18. Albertsson-Wikland K, Isaksson O. Development of responsiveness of young normal rats to growth hormone. Metabolism **25**:747–759, 1976.
19. Goodman HM, Schwartz J. Growth hormone and lipid metabolism. In: Greep RO, Astwood FG, Geiger SR, Eds. Handbook of Physiology. Washington DC: American Physiology Society, pp211–231, 1974.

20. Schwartz J. Enhanced sensitivity to insulin in rats treated with antibodies to rat growth hormone. Endocrinology **107**:877–883, 1980.
21. Goodman HM, Coiro V. Induction of sensitivity to the insulin-like action of growth hormone in normal rat adipose tissue. Endocrinology **108**:113–119, 1981.
22. Coiro V, Grichting G, Goodman HM. Induction of insulin-like responses to growth hormone by stress. Endocrinology **109**:2213–2219, 1981.
23. Grichting G, Levy LK, Goodman HM. Relationship between binding and biological effects of human growth hormone in rat adipocytes. Endocrinology **113**:1111–1120, 1983.
24. Gause I, Eden S, Isaksson O, DiGirolamo M, Smith U. Changes in growth hormone binding and metabolic effects of growth hormone in rat adipocytes following hypophysectomy. Acta Physiol Scand **124**:229–235, 1985.
25. Grichting G, Goodman HM. Growth hormone maintains its own receptors in rat adipocytes. Endocrinology **119**:847–854, 1986.
26. Eden S, Schwartz J, Kostyo JL. Effects of preincubation on the ability of rat adipocytes to bind and respond to growth hormone. Endocrinology **111**:1505–1512, 1982.
27. Salem MAM, Phares CK. Insulin-like effects in the rat of the purified growth factor from *Spirometra mansonoides* plerocercoids. Proc Soc Exp Biol Med **185**:31–38, 1987.
28. McLeay MT, Phares CK. Purification of the growth hormone analogue produced by the tapeworm, *Spirometra mansonoides*. Fed Proc **43**:631, 1984.
29. Holloway PW. A simple procedure for removal of Triton X-100 from protein samples. Analy Biochem **53**:304–308, 1973.
30. Horigome T, Sugano H. Rapid method for removal of detergents and salts from protein solutions using toyopearl HW-40F. J Chromatography **283**:315–319, 1984.
31. Goodman HM. Early and late effects of growth hormone on the metabolism of glucose in adipose tissue. Endocrinology **76**:1134–1140, 1965.
32. Dole VP. A relation between nonesterified fatty acids in plasma and metabolism of glucose. J Clin Invest **35**:150–154, 1956.
33. Wieland O. Ein enzymatishe methode zur bestimmung von glycerin. Biochem Z **329**:313–319, 1957.
34. Nilsson NO, Belfrage P. Effects of acetate, acetaldehyde, and ethanol on lipolysis in isolated rat adipocytes. J Lipid Res **19**:737–742, 1978.
35. Vaughan M. The production and release of glycerol by adipose tissue incubated *in vitro*. J Biol Chem **237**:3354–3358, 1962.
36. Goodman HM. Separation of early and late responses of adipose tissue to growth hormone. Endocrinology **109**:120–129, 1981.
37. Rodbell M. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem **239**:375–380, 1964.
38. Gliemann J, Osterlind K, Vinten J, Gammeltoft S. A procedure for measurement of distribution spaces in isolated fat cells. Biochem Biophys Acta **286**:1–9, 1972.
39. Goodman HM. Antilipolytic effects of growth hormone. Metabolism **19**:849–855, 1970.
40. Birnbaum RS, Goodman HM. Studies on the mechanism of the antilipolytic effects of growth hormone. Endocrinology **99**:1336–1345, 1976.
41. Birnbaum RS, Goodman HM. Comparison of several insulin-like effects of growth hormone. Horm Metab Res **11**:136–142, 1979.
42. Bjogell P, Rosberg S, Isaksson O, Belfrage P. The antilipolytic effect of growth hormone is caused by a net decrease of hormone-sensitive lipase phosphorylation. Endocrinology **115**:1151–1156, 1984.
43. Chipkin SR, Kostyo JL, Tai LR, Goodman MH. Different growth hormone-receptor interactions produce early insulin-like and late lipolytic responses in rat adipose tissue. 70th Annual Meeting of the Endocrine Society, p84, 1988.
44. Salem MAM, Phares CK. Some biochemical effects of the growth hormone analogue produced by plerocercoids of the tapeworm, *Spirometra mansonoides* on carbohydrate metabolism of adipose tissue from normal, diabetic and hypophysectomized rats. J Parasitol **72**:498–506, 1986.