Porcine Insulin-Like Growth Factor (IGF) Binding Protein Blocks IGF-I Action on Porcine Adipose Tissue[1] (42865)

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Abstract. **A growth hormone-dependent insulin-like growth factor (IGF) binding protein (IGFBP) purified from porcine serum specifically blocked the acute insulin-like effects of IGF-I on lipogenesis and glucose oxidation in porcine adipose tissue. This inhibition was dose dependent with half-maximal effective concentrations of IGFBP of 530 ng/ml for lipogenesis and 590 ng/ml for glucose oxidation in the presence of 10^{-8} *M* IGF-I. The IGFBP also caused decreased rates of lipogenesis following a 1-hr preincubation of tissue with IGF-I (10^{-8} *M*). The IGFBP had no effect on insulin action on porcine adipose tissue.**

These findings demonstrate the inhibitory effects of a highly purified porcine serum IGFBP on the biologic effects of IGF-I *in vitro*, and provide evidence that the growth hormone-dependent IGFBP blocks the acute insulin-like actions of IGF-I *in vivo*.

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Insulin-like growth factor I (IGF-I) is a growth hormone (GH)-dependent mitogenic peptide which shares homology with insulin (1, 2). IGF-I also expresses acute insulin-like actions on the classical insulin target tissues (1). Thus, exogenous IGF-I causes hypoglycemia in pigs (3) and other species (4, 5). It has been hypothesized that the insulin-like effects of IGF-I do not normally occur *in vivo* because IGF-I is bound to insulin-like growth factor binding protein (IGFBP) present in the circulation (6). Evidence to support this hypothesis is provided by two studies in which partially purified IGFBP from human serum abolished the short-term insulin-like effects of IGF-I on heart muscle and adipocytes (7, 8). Furthermore, Knauer and Smith (9) showed that an IGFBP synthesized by cultured rat liver cells blocked the stimulatory effects of multiplication stimulatory activity on glucose transport in fibroblasts.

We have recently purified an acid-stable IGFBP from pig serum which has a molecular weight of 50 kDa and appears to be a subunit of the GH-dependent 150-kDa IGFBP (10). This acid-stable IGFBP specifically binds IGF-I and II and is structurally homologous with IGFBP recently purified from rat serum and human plasma (3, 10–12). We are interested in the properties of this porcine IGFBP and its role in IGF-I and GH action in pigs. In the current study we determined the effects of porcine acid-stable IGFBP on the acute insulin-like effects of IGF-I in pig adipose tissue. This is the first demonstration of the effects of a highly purified IGFBP from serum on IGF-I action in adipose tissue *in vitro*. We have previously found that porcine adipose tissue is sensitive to the acute insulin-like effects of IGF-I (13) which provides a suitable model system to further assess the effects of the porcine IGFBP.

Materials and Methods

Materials. Porcine insulin was donated by Dr. R. Chance (Eli Lilly and Co., Indianapolis, IN). Recombinant human IGF-I was donated by Dr. B. D. Burleigh (Pitman-Moore, Northbrook, IL). Hepes was purchased from United States Biochemical Corp. (Cleveland, OH). Bovine serum albumin was purchased from Armour Pharmaceutical Co. (Kankakee, IL). D-[U-^{14}C]Glucose was purchased from New England Nuclear (Boston, MA). Osmium tetroxide was purchased from Electron Microscopy Services (Fort Washington, PA). All other chemicals were reagent grade and were purchased from VWR Scientific (Philadelphia, PA).

Adipose Tissue Incubations. Explants (0.4-mm thick, approximately 10 mg) were prepared from subcutaneous adipose tissue samples obtained from biopsy

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of a 75-kg pig (male castrate) as described previously (14). Explants were incubated in 3 ml of Kreb's Ringer bicarbonate buffer containing 25 mM Hepes, 3% w/v bovine serum albumin, 1.0 μCi of D-[U-^{14}C]glucose (pH 7.4), and various concentrations of IGF-I or insulin at 37°C for 2 hr. Lipogenesis and glucose oxidation were determined by measuring incorporation of labeled glucose into lipid and CO_2, and adipocyte number was determined as described previously (14). IGFBP was included in certain experiments to assess its effects on IGF-I- and insulin-stimulated lipogenesis and glucose oxidation.

Dose-response curves were fitted to the data using the ALLFIT program (15), and differences between hormone and IGFBP treatment means were analyzed using Student's t test (16).

Results

As noted previously (13), lipogenesis and glucose oxidation in pig adipose tissue are stimulated in a dose-dependent manner by insulin and IGF-I (Fig. 1). The EC_{50} for the insulin response curves were 4.2×10^{-11} and 2.3×10^{-11} M for lipogenesis and glucose oxidation, respectively. The EC_{50} for the IGF-I curves were 1.5×10^{-9} and 6.9×10^{-9} M for lipogenesis and glucose oxidation, respectively.

Explants were incubated with IGF-I (10^{-8} M) or insulin (1.7×10^{-10} M) in the presence and absence of IGFBP (1 μg/ml) to evaluate IGFBP effects on insulin and IGF-I action (Fig. 2). The concentrations of insulin and IGF-I were chosen to given approximately equivalent, maximal stimulation of lipogenesis and glucose oxidation. Lipogenesis in the presence of insulin with and without IGFBP was significantly greater than basal rates in the absence of any hormone (Fig. 2). Although it appears that rates of lipogenesis in the presence of both IGFBP and insulin are greater than with insulin alone, these values are not significantly different when compared using Student's t test ($P < 0.05$). In contrast, IGFBP blocked IGF-I-stimulated lipogenesis such that metabolic rates were not different from basal values (Fig. 2). The situation is similar for glucose oxidation data (Fig. 2), except that the values for insulin alone had more variability and therefore were not significantly different from basal rates ($P < 0.05$). However, it should be noted that the mean glucose oxidation in tissue incubated with insulin alone also was not different from stimulated rates in the presence of insulin or IGF-I in combination with IGFBP ($P < 0.05$). As was the case with lipogenesis, IGFBP blocked the stimula-

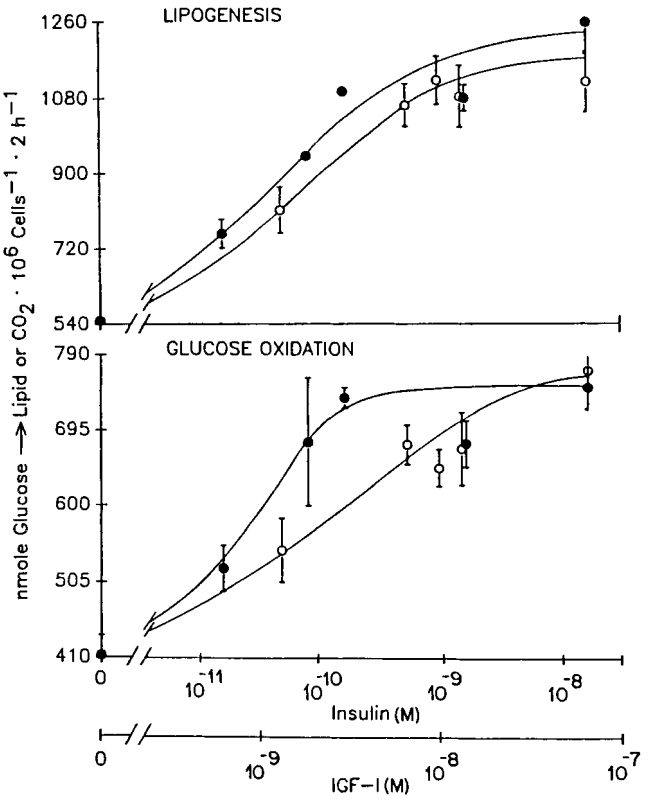

Figure 1. The response curves for lipogenesis and glucose oxidation in the presence of increasing concentrations of insulin (●) and IGF-I (○). The EC_{50} determined using ALLFIT (13) were 4.2×10^{-11} and 1.5×10^{-9} M (lipogenesis) and 2.3×10^{-11} and 6.9×10^{-9} M (glucose oxidation) for insulin and IGF-I, respectively. Values are mean ± SE for $n = 3$ observations.

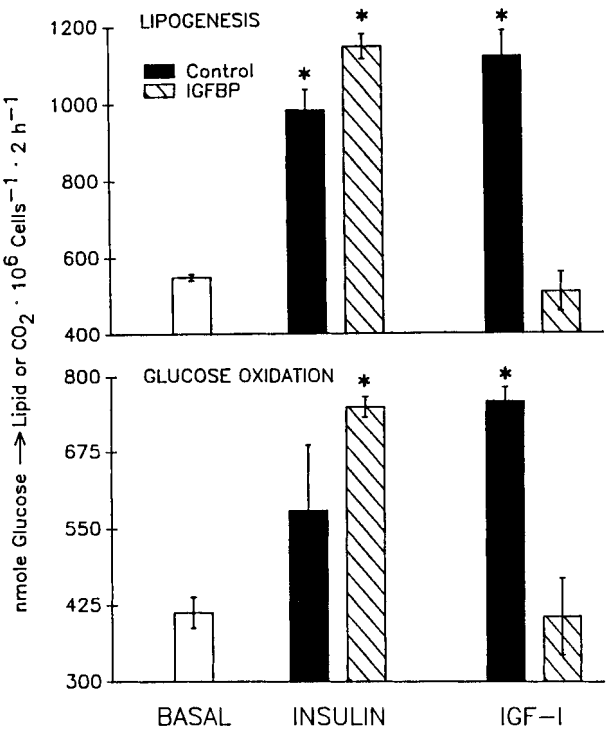

Figure 2. Comparison of insulin- and IGF-I-stimulated lipogenesis and glucose oxidation in the presence and absence of IGFBP (1 μg/ml). Insulin concentration was 1.7×10^{-10} M; IGF-I concentration was 10^{-8} M. Basal rates of lipogenesis and glucose oxidation were measured in the absence of any hormone. Values are mean ± SE for $n = 3$ observations. Differences between treatment means and the basal value were tested using Student's t test (*P<0.05).

tory effects of IGF-I on glucose oxidation in pig adipose tissue (Fig. 2).

The dose-response relationship for the inhibition of IGF-I-stimulated lipogenesis and glucose oxidation by IGF-I was examined by incubating adipose tissue with increasing concentrations of IGFBP in the presence of a fixed level (10^{-8} *M*) of IGF-I (Fig. 3). A dose-dependent inhibition of each parameter was observed with EC_5 of 530 and 590 ng/ml for lipogenesis and glucose oxidation, respectively.

To test whether IGFBP affected lipogenesis in tissue preincubated with IGF-I, tissue was incubated for up to 1 hr in the presence of 10^{-8} *M* IGF-I, then IGFBP (1 μg/ml) was added to some incubation vials, and the incubations were continued for an additional 2 hr (Fig. 4). The added IGFBP caused a 31% decline in lipogenic rate.

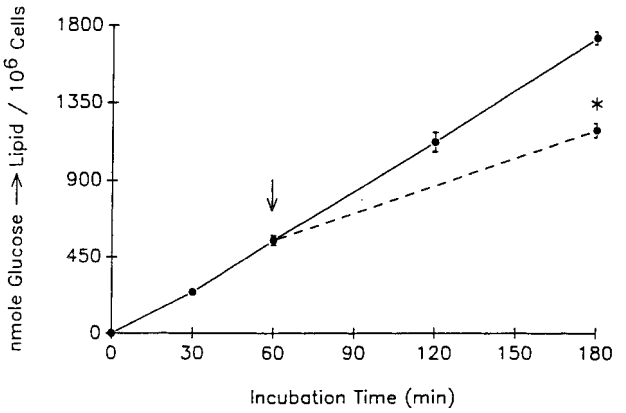

Figure 4. Rates of IGF-I-stimulated lipid synthesis over 3 hr. Following 1-hr preincubation, IGFBP (1 μg/ml) was added (indicated by arrow). IGF-I concentration = 10^{-8} *M*. The rate of lipid synthesis in the presence of IGFBP (– – –) was significantly less than with IGF-I alone (*Student's *t* test, $P<0.05$).

Discussion

The current study confirms our previous observation that pig adipose tissue is very sensitive to the stimulatory effects of insulin and IGF-I on lipogenesis and glucose oxidation (13). Insulin was more potent in stimulating adipose tissue than IGF-I; the potency ratio of insulin to IGF-I in the current study was 1:36 and 1:300 for lipogenesis and glucose oxidation, respectively, which are similar in magnitude to that observed for glucose utilization by rat adipocytes (1:110; 17). These insulin to IGF-I potency ratios are consistent with the hypothesis that IGF-I produces acute insulin-like effects in adipocytes via the insulin receptor and not via the Type I IGF receptor (18).

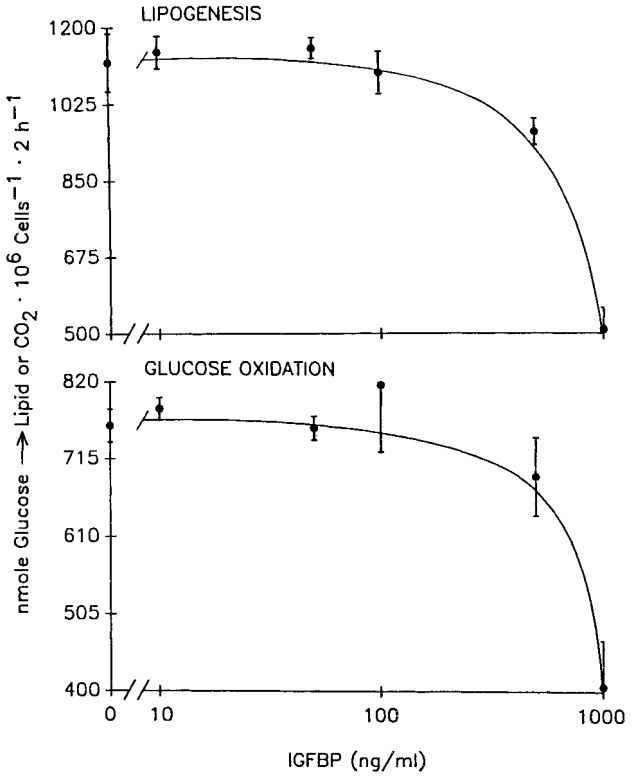

Figure 3. The effect of increasing concentrations of IGFBP on IGF-I-stimulated lipogenesis and glucose oxidation. IGF-I concentration = 10^{-8} *M*. The EC_{50} determined using ALLFIT (13) were 530 and 590 ng/ml for lipogenesis and glucose oxidation, respectively. Values are mean ± SE for $n = 3$ observations.

The acid-stable porcine IGFBP blocks the acute insulin-like effects of IGF-I on pig adipose tissue. This effect of IGFBP is specific for IGF-I since insulin action was not affected. It should be noted, however, that we have only tested IGF-I and insulin and not any other growth factors. We examined these effects of the IGFBP on insulin and IGF-I action at close to the minimal concentration of each hormone which elicited maximal stimulation of glucose metabolism to avoid discrepant results due to an excess (or deficit) of either hormone. The inhibitory effects of IGFBP were dose dependent, and it is interesting to note that the EC_{50} for this inhibition are approximately equimolar to the concentration of IGF-I in the incubation buffer, i.e., for lipogenesis 530 ng/ml = 1.06×10^{-8} *M* IGFBP and for glucose oxidation 590 ng/ml = 1.18×10^{-8} *M* IGFBP (calculated using an approximate molecular weight of 50 kDa for IGFBP) vs an IGF-I concentration of 10^{-8} *M*. This molar ratio suggests a 1:1 binding relationship between the IGFBP and IGF-I. It should also be noted that the inhibitory effects of IGFBP observed in this study occurred with IGFBP concentrations which we have determined using a specific radioimmunoassay for the IGFBP to be in the physiologic range (i.e., 0.5–1.5 μg/ml; 3, 11). This clearly suggests that the IGFBP functions *in vivo* to suppress the acute insulin-like actions of IGF-I. In addition, these observations support those of Cornell *et al.* (19) that most of the IGF-I serum is bound to an acid-stable, metabolically inactive complex.

The observation that addition of IGFBP to adipose

tissue previously incubated with IGF-I caused a marked decrease in lipid synthesis (Fig. 4) suggests that the binding protein sequesters IGF-I from receptors on adipocytes as well as blocks binding to IGF-I in the buffer to binding sites on the adipocyte. Thus, the rates of lipid synthesis decline after 2 hr of incubation with both IGFBP and IGF-I. This postulated interaction between IGFBP, IGF-I, and the Type I IGF receptor is supported by recent findings which indicate that IGF-I and II binding to cultured porcine aortic endothelial cells is blocked by the pig IGFBP (20). Furthermore, the addition of IGFBP to porcine aortic endothelial cells incubated with ^{125}I-IGF-I causes a marked dissociation of bound IGF-I which suggests that the IGFBP can sequester IGF-I bound to the receptor. Although we have not examined ^{125}I-IGF-I binding to pig adipocytes in the presence of IGFBP, it is our speculation based on the results of the adipose tissue incubations and the porcine aortic endothelial cells binding studies that the pig IGFBP has a higher affinity than adipocyte receptors for IGF-I.

An acid-stable IGFBP purified from pig serum that is a component of the 150-kDa GH-dependent IGFBP specifically blocks the acute insulin-like effects of IGF-I in pig adipose tissue. Recently, DeMellow and Baxter (21) found that co-incubation of IGF-I and human acid-stable IGFBP also inhibited DNA synthesis in human skin fibroblasts. It is yet to be ascertained whether the intact 150-kDa IGFBP in serum also blocks these effects of IGF-I. Since the acid-stable IGFBP likely comprises the IGF binding site of the larger complex (22), however, one interpretation of these findings is that the 150-kDa IGFBP also blocks the insulin-like effects of IGF-I in adipose tissue and the mitogenic effects observed in proliferating cells. Other evidence suggests that this interpretation is open to question. DeMellow and Baxter (21) also noted that if fibroblasts were preincubated with the acid-stable IGFBP prior to the addition of IGF-I that the effects of IGF-I on DNA synthesis were potentiated by the IGFBP. This response is in contrast to the inhibitory effects observed when IGF-I and the IGFBP were co-incubated. It can be argued that since there is no free detectable IGF-I in serum that the physiologically relevant *in vitro* approach is to co-incubate IGF-I with the IGFBP. However, there are uncertainties about this idea since it has been established that fibroblasts synthesize and secrete IGFBP into the culture medium that enhance binding and the biologic effects of IGF-I *in vitro* (23–25). It is evident that further work is necessary to resolve the biologic roles of the different IGFBP and the role that they play in modulating the insulin-like effects observed in adipose tissue and the mitogenic effects observed in proliferating cells. It should also be recognized that the actions of the IGFBP on the insulin-like effects of IGF-I in adipose tissue may differ from those on cell proliferation.

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