

Plasma Concentrations of Hormones and Metabolic Substrates in Growing Pigs after Bovine Growth Hormone Injections under Various Nutritional Conditions

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Abstract. The effects of GH injections on the concentrations of plasma hormones and metabolic substrates in pigs were studied under various nutritional conditions. In experiment 1, four pigs (Large white, barrows, 108 days old) were maintained at three feeding levels: (1) high level ($8,501 \pm 46$ kcal/day), (2) maintenance level ($2,104 \pm 44$ kcal/day), and (3) fasting (last meal was on day -2). The bovine GH ($100 \mu\text{g/kg BW}$) was subcutaneously injected on experiment days 0, 1, and 2. In experiment 2, four pigs (Landrase, barrows, 120 days old) were maintained at the high feeding level and the bovine GH ($100 \mu\text{g/kg BW}$) was injected 2, 26, and 50 h after the last meal. The plasma hormone and metabolic substrate concentrations were measured. In experiment 1, the plasma IGF-I concentrations increased on days 1–4 in the high feeding level and maintenance fed pigs, but did not increase in the fasting pigs. The plasma glucose concentrations increased after the GH injection in the high feeding level and maintenance fed animals. The plasma NEFA concentrations increased after the GH injection in the maintenance fed and fasting animals. In experiment 2, the plasma IGF-I gradually decreased after the last meal. The GH injection administered 26 or 50 h after the last meal still produced an increase in the plasma IGF-I levels. These data clearly show that the effect of GH was modified depending on the nutritional condition of the pigs.

Key words: GH, Nutrition, IGF-I, Metabolites, Pig

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THE DAILY administration of GH stimulates growth in normal pigs [1–3]. The 36% faster growth rate and 28% lower feed: gain ratio were reported during the treatment period from 30 kg to 60 kg with reduced carcass fat and increased carcass protein [3]. It has been considered that most of the GH effects are mediated by insulin-like growth factor-I (IGF-I). The effects of the administered GH may be mediated by the IGF-I in pigs. Plasma levels of IGF-I are influenced by the nutritional status of pigs [4] as well as dogs and cattle [5, 6].

However, there is little information so far indicating how the administered GH effects the endocrine status under the various nutritional conditions of pigs. It is also reported that the response of plasma IGF-I to administered GH is decreased or suppressed by reduced feed intake for 3 days in cattle [6] or fasting for 5 days in human [7]. How the response of GH is changed during the first three days in fasting is still not clear in pigs and other species.

In the present study, the effect of the GH administration on the plasma IGF-I concentrations in growing pigs was investigated under three levels of feedings. The plasma insulin, thyroid hormones, glucose, and non-esterified fatty acid (NEFA) concentrations were measured. We also investigated the responsiveness of IGF-I to exogenous GH dur-

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ing 72 h fasting.

Materials and Methods

Animals and experimental designs

Experiment 1: Effect of GH at various feeding levels

Four pigs (Large white, barrows, 108 days old, 49.9 ± 1.0 kg body weight (BW) at the beginning of the experiments) were surgically fitted with jugular vein catheters and maintained at 20 °C in an air-conditioned room. The pigs were fed at three feeding levels: (1) high level, (2) maintenance level, and (3) fasting level. At the high level, the pigs were fed $8,501 \pm 46$ kcal/day and 357 ± 1.9 g/day of digestive crude protein (DCP). At the maintenance level, the pigs were fed $2,104 \pm 44$ kcal/day and 88 ± 1.9 g/day of DCP. The amount of feeding was calculated according to the Japanese Feeding Standard for Swine [8]. The diet for fattening the pigs, which was formulated by our institute, was used throughout the experiments (TDN 70.1%, DCP 12.7%). These pigs were fed half the volume mentioned above at 0900 h and 1700 h. In the fasting experiment, the pigs were fed at high levels until the last meal, which was given at 0900 h on day -2.

Every day at 0900 h before feeding during the experiment, blood samples were taken through the catheter. At 1100 h on days 0, 1, and 2, 100 µg/kg BW of recombinant bovine GH (Eli Lilly Co.) dissolved in 10 ml of carbonate buffer (pH = 9.5) was subcutaneously injected. Blood samples on day 1 were taken at -2, 0, 1, 2, 3, 4, 5, 6, 12, and 24 h relative to the injection. The samples were placed in tubes containing heparin and were immediately chilled on ice until centrifugation. The plasma samples were stored at -80 °C prior to the hormone assays.

Experiment 2: Effect of GH under different durations of fasting

Four pigs (Landrace, barrows, 120 days old, 50.4 ± 0.7 kg BW) were surgically fitted with jugular vein catheters. The pigs were fed at the high level until feeding was stopped. The last meal was given at time 0. At the times 2 h, 26 h, and 50 h, 100 µg/kg BW of the bovine GH or vehicle was subcutaneously injected and blood samples were taken

at -2, 0, 1, 2, 3, 4, 5, 6, 9, 12, 22, and 24 h relative to the injection. The samples were treated as previously mentioned.

Plasma hormone assays

To determine the basal level of the plasma GH concentrations, a porcine GH (pGH) radioimmunoassay (RIA) was employed, which was described elsewhere [9]. Concentrations less than 1 ng/ml were calculated as 1 ng/ml in further analyses.

To determine the plasma GH concentrations after the GH injections, a bovine GH (bGH) RIA was employed, which was described previously [10].

The plasma IGF-I was extracted with acid ethanol, and its concentration was determined by RIA as previously described [11]. The plasma T_3 and T_4 were determined by RIA as previously mentioned [12]. The plasma insulin concentrations were determined with a specific RIA kit (Eiken Chemical Co.).

Plasma metabolite assays

The plasma glucose and NEFA concentrations were determined with specific assay kits (Glucose B-test Wako and NEFA-test Wako, respectively; Wako Pure Chemical Co.).

Statistical analyses

The statistical analyses were performed using the statistical software package, SAS (release 6.09.02, 1994, by SAS Institute). The means and standard error (SEM) of the hormone and metabolite concentrations were calculated using the means procedure of SAS and expressed as the mean \pm SEM. Data in experiment 1 were subjected to an analysis of variance (ANOVA) using the GLM procedure. The model included feeding level, day (or time), and animal as the main effects, as well as the interaction between the feeding level and day (or time). Tukey's studentized range test was employed to assess the hormone or metabolite levels between feeding levels. Then the data were sorted by feeding level and subjected to an ANOVA by every feeding level. The model included day (or time) and animal as the main effects. Dunnett's *t*-test was used to assess the hormone and metabolite levels between day 0 (or time 0) and other experimental points at one feeding level. In experiment

2, the paired *t*-test was used to assess the statistical significance of the concentrations between the control and GH injected animals.

Results

Experiment 1: Effect of GH at various feeding levels

The plasma IGF-I and pGH concentrations at 0900 h are shown in Fig. 1. The plasma IGF-I concentrations were elevated in the high level and maintenance fed animals. However, in the case of the fasting animals, the plasma IGF-I levels were not elevated. Plasma IGF-I levels of three groups were different each other. The plasma pGH concentrations were very low after day 1 in all groups. The plasma T_3 concentrations at 0900 h were significantly increased on the day 1–3. However, the plasma T_4 concentrations were significantly de-

creased on the day 4 (Fig. 2). The bovine GH levels in the plasma were elevated within 2 h after the bGH injection and the higher GH levels were continued for at least 4 h. The plasma bGH returned to the basal level within 24 h (data not shown). The changes in the plasma bGH levels after the bGH injection were similar in all of the feeding levels. The plasma glucose concentrations were increased in the animals fed at the high and maintenance levels after the GH injection (Fig. 3 upper). The plasma NEFA concentrations in fasting animals were significantly higher than those in the animals fed at the high and maintenance levels (Fig. 3 middle). The plasma NEFA concentrations were increased after the GH injection in maintenance fed and fasting animals. Plasma NEFA concentrations were not changed in the high level fed animals. Plasma insulin concentrations in the fasting animals were lower than those in the animals fed at the high and maintenance levels (Fig. 3

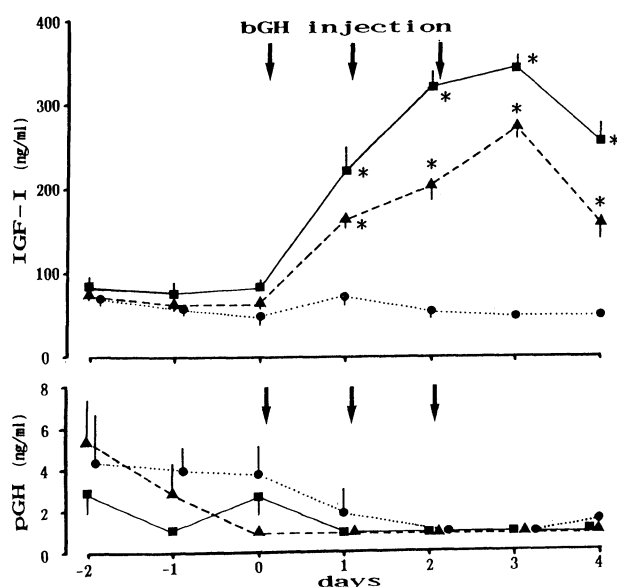


Fig. 1. Effect of GH injections on the plasma IGF-I (upper) and pGH (lower) concentrations at 0900 h in pigs fed at the high level (■), maintenance level (▲), and fasting level (●). The bGH (100 μ g/kg BW) was subcutaneously injected at 1100 h on days 0, 1, and 2. The asterisks indicate the statistical significance of the concentrations compared to pre-injection on day 0 ($P < 0.05$, Dunnett's *t*-test). Plasma IGF-I concentrations in animals at three feeding levels were different from each other (Tukey's studentized range test, $P < 0.05$). Each point with the vertical bar in the graph represents the mean \pm SEM, $n=4$.

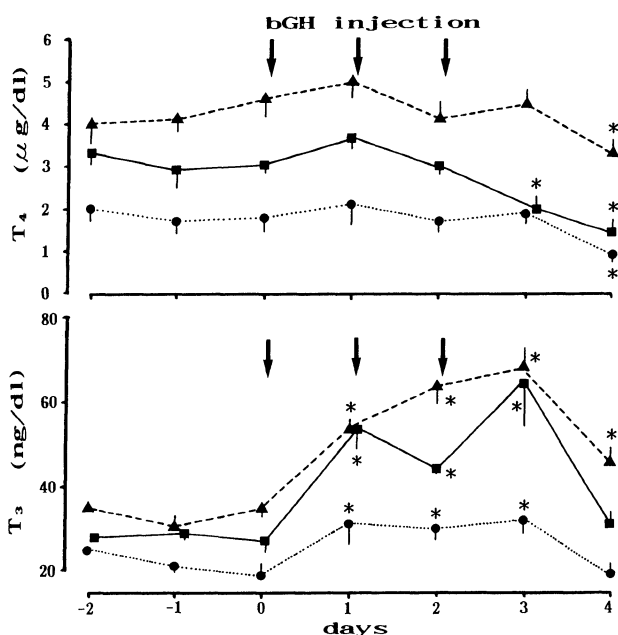


Fig. 2. Effect of GH injections on the plasma T_4 (upper) and T_3 (lower) concentrations at 0900 h in pigs fed at the high level (■), maintenance level (▲), and fasting level (●). The bGH (100 μ g/kg BW) was subcutaneously injected at 1100 h on days 0, 1, and 2. The asterisks indicate the statistical significance of the concentrations compared to pre-injection on day 0 ($P < 0.05$, Dunnett's *t*-test). Plasma T_4 and T_3 levels in animals at three feeding levels were different from each other (Tukey's studentized range test, $P < 0.05$). Each point with the vertical bar in the graph represents the mean \pm SEM, $n=4$.

lower). Plasma insulin concentrations in the animals fed at the maintenance levels were decreased after the GH administration.

Experiment 2: Effect of GH under different durations of fasting

Figure 4 (upper) shows the plasma bGH concentration after the bGH injection during the fasting period. The plasma bGH increases were similar when the injections were performed at 2, 26, or 50 h after the last meal. Figure 4 (lower) shows the change in plasma IGF-I during the fasting period. Plasma IGF-I gradually decreased after the last meal. The GH injection administered 26 h or 50 h after the last meal still had the effect of increasing

plasma IGF-I concentrations. In any case, plasma IGF-I concentrations were significantly increased 12 h after the bGH injection as compared to the control (vehicle injection).

Discussion

The plasma IGF-I concentrations were extremely increased in the high level fed animals, slightly increased in the maintenance fed animals, and not increased in the fasting animals in experiment 1. The reduced responsiveness to the exogenous GH in the low energy intake or the protein-restricted conditions has been reported in several species [6, 7, 13]. However, as the plasma IGF-I concentrations in the control animals were decreasing over 72 h, the responsiveness to the exogenous GH was not lost or extremely reduced even in the fasting

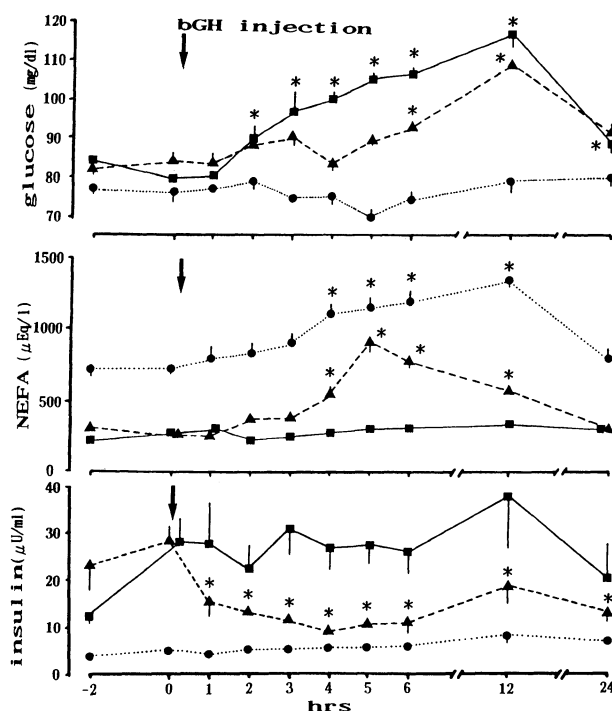


Fig. 3. The plasma glucose (upper), NEFA (middle), and insulin (lower) concentrations after the GH injection on day 1 in the pigs fed at the high level (■), maintenance level (▲), and fasting level (●). The bGH (100 μ g/kg BW) was subcutaneously injected at time 0. The asterisks indicate the statistical significance of the concentrations compared to pre-injection at time 0 ($P < 0.05$, Dunnett's *t*-test). Plasma glucose, NEFA, and insulin concentrations in three feeding levels were different from each other (Tukey's studentized range test, $P < 0.05$). Each point with the vertical bar in the graph represents the mean \pm SEM, $n = 4$.

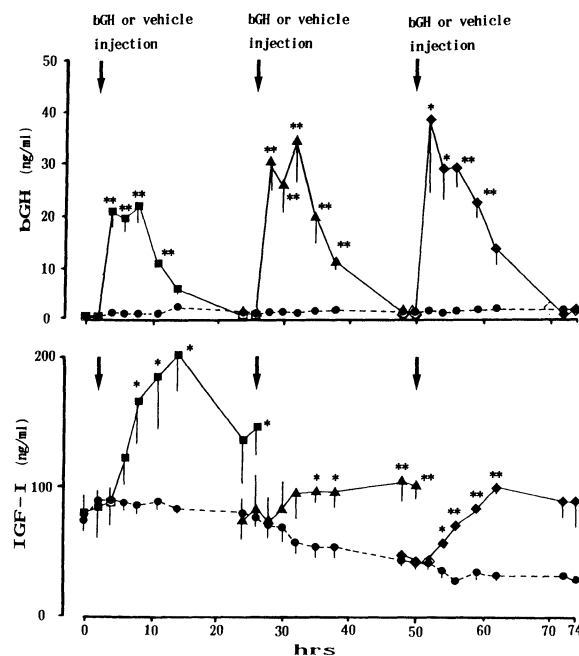


Fig. 4. Effect of GH injection on the plasma bGH (upper) and IGF-I (lower) concentrations in control pigs (vehicle injected: ●) and bGH injected pigs (100 μ g/kg BW, ■ at time 2 h, ▲ at time 26 h and ◆ at time 50 h) under the different durations of fasting. The last meal was given at time 0. The asterisks indicate the statistical significance between the control and GH injected animals (*: $P < 0.05$, **: $P < 0.01$, the Student's paired *t*-test). Each point with the vertical bar in the graph represents the mean \pm SEM, $n = 4$.

animals (Fig. 4). On the other hand, the repetitive GH injections increased plasma IGF-I concentrations, even though the plasma IGF-I concentrations had already been high when nutritional conditions were fair. It is reported that the repeated GH administrations were more effective in increasing plasma IGF-I in normal pigs than a single bolus administration [14].

The secretion of endogenous GH (pGH) seemed to be suppressed by the GH administration (Fig. 1 lower), because all the animals ($n=4$) in experiment 1 had low pGH concentrations (less than 1 ng/ml) after the GH administration, whilst 1 or 2 animals always had high pGH concentrations before GH administration. However, this suppression of GH secretion was not statistically significant because of the large individual variation in plasma pGH concentrations. And in experiment 1, only one sample was obtained from each animal on each experimental day. It was difficult to determine the GH secretion status in the animals by this sampling schedule, because GH is secreted in a pulsatile manner.

The plasma T_3 concentrations were increased after the GH injections (Fig. 2). The increase in T_3 after GH administration was in agreement with a previous report in pig [15]. It is seemed that the increase in T_3 is not mediated at the level of the thyroid gland, because it was reported that the effects of thyrotropin stimulating hormone were not different between GH-treated pigs and controls [16]. And it was reported that the activity of hepatic 5' monodeiodinase was increased after GH administration in chickens [17]. This enzyme converts T_4 to T_3 . The increase in plasma T_3 after GH injection in this study may reflect the T_4 to T_3 converting activity in peripheral tissue. And the increase in plasma T_3 in fasting pigs was smaller than that in high level or maintenance fed pigs, which may also reflect the change in deiodinase activity influenced by the nutritional status of the pigs.

The administration of the GH increased the plasma glucose concentrations in the high level and maintenance fed animals; however, the plasma glucose concentrations in the fasting animals were not increased (Fig. 3 upper). In contrast, the plasma NEFA concentrations were increased in the fasting and maintenance fed animals but not elevated in the high level fed animals (Fig. 3 middle). These data suggest that the glucose and NEFA utiliza-

tion after the GH injection changed with the nutritional conditions of the animals.

It is well-known that GH has a diabetogenic effect. In this study, in the high level and maintenance fed animals, the plasma glucose concentrations were increased after GH injection as expected. Plasma glucose concentrations were the highest at 12 h after the GH injection. On the other hand, plasma GH concentrations were the highest at 2–6 h after the GH injection. Apparently this seems to be a discrepancy. However, according to a recent report, an increase in the plasma glucose concentrations was observed during IGF-I infusion into sheep [18]. At the same time, a decrease in the plasma insulin concentration was observed. A decrease in the plasma insulin concentration during an IGF-I infusion was also reported in human [19]. In this study, a decrease in the plasma insulin concentration was observed in the maintenance fed pigs (Fig. 3 lower). And despite the highest glucose concentration 12 h after GH injection, no significant increase in the plasma insulin concentration was observed in the high level or maintenance fed animals. According to these evidences, it is seemed that a direct effect of GH may increase the plasma glucose level at first, then an insufficient insulin secretion caused by an increase in plasma IGF-I may produce an additional increase in plasma glucose.

Plasma NEFA concentrations were increased in fasting and maintenance fed animals (Fig. 3 middle). It is reported that GH can alter the responsiveness of adipose tissue to other lipolytic hormones. GH treatment increased the response to a catecholamine challenge in lactating cows [20]. In this study, it was seemed that the fasting animals with low glucose concentrations might have high catecholamine concentrations. And low insulin and IGF-I concentrations in fasting animals also promoted lipolysis in adipose tissue. Under these conditions, GH treatment may have increased plasma NEFA concentrations in fasting animals. On the other hand, in maintenance fed animals, plasma insulin concentrations were decreased after the GH injection. This decrease in plasma insulin may have increased plasma NEFA.

In conclusion, the changes in plasma IGF-I, T_3 , glucose, and NEFA concentrations after the GH injection differed depending on the nutritional condition of the pigs. Although the plasma IGF-I concentrations did not increase in the fasting ani-

mals, GH still had an effect in increasing or maintaining the plasma IGF-I concentrations, because plasma IGF-I was constantly decreasing in the control animals. These data clearly show that the effect of GH was modified by the nutritional condition of pigs.

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