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Unexpected role for IGF-1 in starvation: Maintenance of blood glucose

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Wild-type (WT) mice maintain viable levels of blood glucose even when adipose stores are depleted by 6 d of 60% calorie restriction followed by a 23-h fast (hereafter designated as "starved" mice). Survival depends on ghrelin, an octanoylated peptide hormone. Mice that lack ghrelin suffer lethal hypoglycemia when subjected to the same starvation regimen. Ghrelin is known to stimulate secretion of growth hormone (GH), which in turn stimulates secretion of IGF-1 (insulin-like growth factor-1). In the current study, we found that starved ghrelin-deficient mice had a 90% reduction in plasma IGF-1 when compared with starved WT mice. Injection of IGF-1 in starved ghrelin-deficient mice caused a twofold increase in glucose production and raised blood glucose to levels seen in starved WT mice. Increased glucose production was accompanied by increases in plasma glycerol, fatty acids and ketone bodies, and hepatic triglycerides. All of these increases were abolished when the mice were treated with atglistatin, an inhibitor of adipose tissue triglyceride lipase. We conclude that IGF-1 stimulates adipose tissue lipolysis in starved mice and that this lipolysis supplies energy and substrates that restore hepatic gluconeogenesis. This action of IGF-1 in starved mice is in contrast to its known action in inhibiting adipose tissue lipase in fed mice. Surprisingly, the ghrelin-dependent maintenance of plasma IGF-1 in starved mice was not mediated by GH. Direct injection of GH into starved ghrelin-deficient mice failed to increase plasma IGF-1. These data call attention to an unsuspected role of IGF-1 in the adaptation to starvation.

ghrelin-deficient mice | hypoglycemia | IGF-1 | growth hormone-releasing hormone | adipose tissue lipolysis

Significance

Survival in starvation requires maintenance of blood glucose at levels that support vital functions. When mice that lack the hormone ghrelin are starved, they develop lethal hypoglycemia. Here, we show that hypoglycemia in fasted, ghrelin-deficient mice is associated with profound reductions in the plasma level of insulin-like growth factor-1 (IGF-I). Hypoglycemia is reversed by injection of IGF-1, which activates lipolysis in adipose tissue, releasing fatty acids and glycerol that are used by the liver to synthesize glucose. This new function of IGF-1 in starvation is opposite to its long-known antilipolytic, glucose-lowering action when administered to fed animals. These data expose a previously unappreciated role of IGF-1 in survival during periods of famine.

When humans or other animals are subjected to caloric restriction, they exhibit profound decreases in adipose tissue and other energy stores. Despite this loss, calorie-restricted animals can still survive an acute fast. Studies in our laboratory (1–3) and others (4–6) have shown in mice that this survival depends on a unique octanoylated polypeptide hormone, ghrelin.

In our experimental model of famine, mice were placed on diets that contained only 40% of the calories that they would normally consume (i.e., 60% calorie restriction). The mice became ravenous and consumed all of their allotted food within 60 min of feeding each evening at 6:00 PM. They then fasted until their next meal 23 h later. By day 6 of this food restriction, the mice had lost ∼30% of body weight and ∼82% of their body fat (1). Despite this loss, WT mice maintained viable blood glucose levels greater than 40 mg/dL when measured at 5:30 PM after the 23-h fast. Plasma levels of ghrelin increased progressively with each day of calorie restriction. Genetically engineered mice that lack ghrelin could not maintain their blood glucose at levels that support life. Tracer studies showed that their hypoglycemia results from a reduction in glucose production (7). Blood glucose levels were restored to normal when the starved ghrelin-deficient mice were injected with compounds that provide energy, including pyruvate, amino acids, and medium chain fatty acids (7). The data thus indicate that hypoglycemia in ghrelin-deficient mice is caused by a lack of energy and substrates needed to support glucose production. The present paper addresses the mechanism by which ghrelin increases the provision of energy to the liver to support glucose production in fat-depleted mice.

Ghrelin was discovered in 1999 as a uniquely octanoylated peptide that acts upon the growth hormone (GH) secretagogue receptor (GHS-R) in the anterior pituitary to stimulate release of GH (8). Later, it was found that ghrelin also stimulates the secretion of growth hormone-releasing hormone (GHRH) in the hypothalamus (9–11). Like ghrelin itself, GHRH acts on the GHRH receptor in the pituitary to stimulate GH release. The balance between the direct and indirect actions of ghrelin on GH secretion is complex and not well understood (reviewed in ref. 12).

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Ghrelin action absolutely requires octanoate, which is attached to a serine by an endoplasmic reticulum enzyme called ghrelin *O*-acyl transferase (GOAT) (13, 14). Genetically engineered mice that lack GOAT (*Goat*$^{-/-}$ mice) fail to produce active ghrelin. In calorie-restricted WT mice, the increase in plasma ghrelin leads to an increase in plasma GH. This increase in GH is blunted in *Goat*$^{-/-}$ mice (1). Hypoglycemia in calorie-restricted *Goat*$^{-/-}$ mice is prevented when ghrelin or GH is administered chronically through an implanted minipump, beginning with the initiation of calorie restriction (1). However, GH does not increase the blood glucose when its administered acutely during the fasting period after 6 or 7 d of calorie restriction (15). This failure occurs despite data showing that the injected GH is active in the liver, as indicated by induced phosphorylation of STAT5 and induction of autophagy (15, 16).

In addition to stimulating secretion of GH, GHRH also increases plasma levels of insulin-like growth factor-1 (IGF-1). This increase is thought to be mediated by GH, which stimulates IGF-1 mainly in the liver (12, 17). However, 20% of IGF-1 is produced in nonhepatic tissues, including adipose tissue and muscle, where it is thought to have autocrine and paracrine actions (17–19). The factors that control the extrahepatic secretion of IGF-1 are unknown.

In the present study, we demonstrate that acute injection of GHRH raises blood glucose levels in GOAT-deficient mice after 6 d of calorie restriction. Remarkably, the effect is not mediated by the increased GH. Rather, it is attributable to a major increase in plasma IGF-1.

Results

Fig. 1*A* shows blood glucose levels in mice that were subjected to 60% calorie restriction for 6 d followed by 23 h of fasting. The data were pooled from two identical experiments. Despite calorie restriction and fasting, WT mice maintained viable levels of blood glucose with an average of 66 mg/dL. In contrast, *Goat*$^{-/-}$ mice exhibited severe hypoglycemia (mean glucose, 36 mg/dL). Hypoglycemia was prevented when the *Goat*$^{-/-}$ mice were injected with GHRH 2 h prior to being killed (mean glucose, 56 mg/dL). As observed previously (1, 3), plasma GH was reduced in the *Goat*$^{-/-}$ mice (Fig. 1*B*). Injection of GHRH raised plasma GH to levels above that in the WT mice, and GH injection raised the level even higher. Yet, the GH injection failed to raise blood glucose. Thus the glucose-raising effect of GHRH must be attributable to something other than GH.

To explore the mechanism for glucose elevation by GHRH, we measured plasma IGF-1, another protein whose secretion is known to be increased by GHRH (12). As shown in Fig. 1*C*, calorie-restricted *Goat*$^{-/-}$ mice had a profound reduction in total plasma IGF-1 as revealed by the Crystal Chem ELISA that measures both free and protein-bound IGF-1 (20). Remarkably, in the *Goat*$^{-/-}$ mice plasma IGF-1 was not increased by injection of GH despite the fact that GH is considered the normal inducer of IGF-1 synthesis in the liver. On the other hand, IGF-1 was raised dramatically by injection of GHRH. Fig. 1*D* shows that *Goat*$^{-/-}$ mice had a reduced level of phosphorylated STAT5 in the liver, which is consistent with their low plasma GH. Phosphorylated

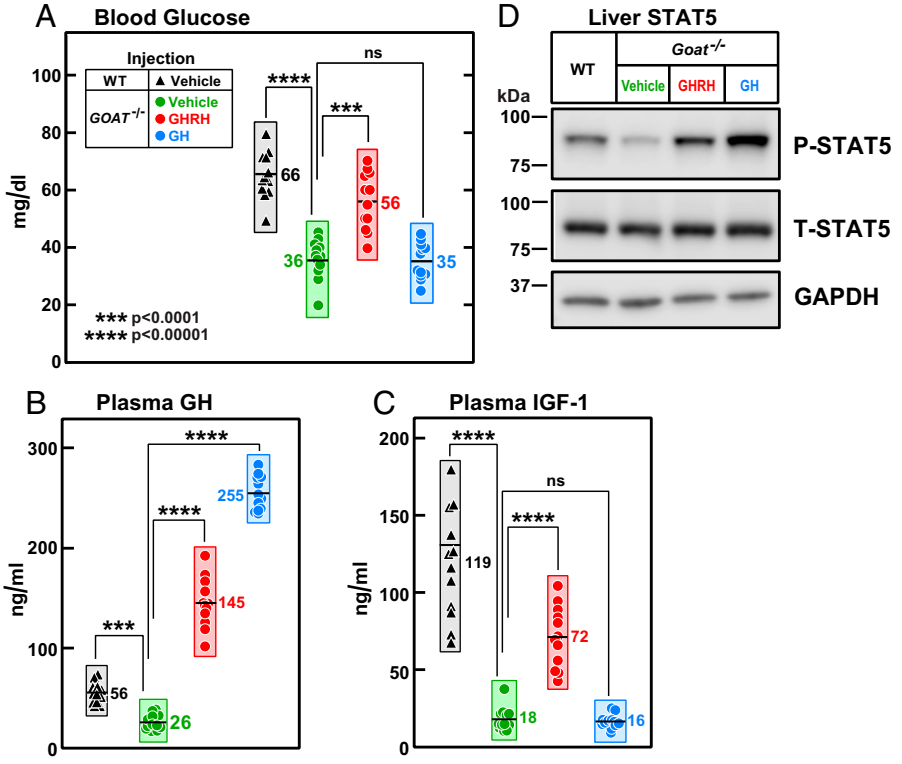

Fig. 1. Differential response of blood glucose levels in calorie-restricted *Goat*$^{-/-}$ mice injected with GHRH or GH. Male WT and *Goat*$^{-/-}$ littermates (8-wk old) were housed individually and subjected to 60% calorie restriction for 6 d as described in *SI Appendix*. On day 6 at 4:00 PM, WT mice were injected intraperitoneally with vehicle, and *Goat*$^{-/-}$ mice received one injection of vehicle, GHRH (10 μg/g body weight), or GH (3 μg/g body weight) as indicated. (*A*) At 5:30 PM, blood was obtained for glucose measurements as described in *SI Appendix*. (*B–D*) At 6:00 PM, the mice were anesthetized, and blood was collected for measurement of plasma levels of GH (*B*) and IGF-1 (*C*), as described in *SI Appendix*. The data shown in *A–C* are from two independent experiments. Numbers beside the data points in *A–C* denote mean values. Asterisks (*) denote levels of statistical significance (Student's *t* test). ns, no significance; ***$P < 0.0001$; ****$P < 0.00001$. (*D*) Immunoblot analysis is shown for one of the two experiments. Equal amounts of liver lysate from six individually prepared samples in each group were pooled, and an aliquot of the resulting pooled supernatant (40 μg) was denatured and loaded onto a SDS gradient gel for detection of p-STAT5, t-STAT5, and GAPDH, as described in *SI Appendix*.

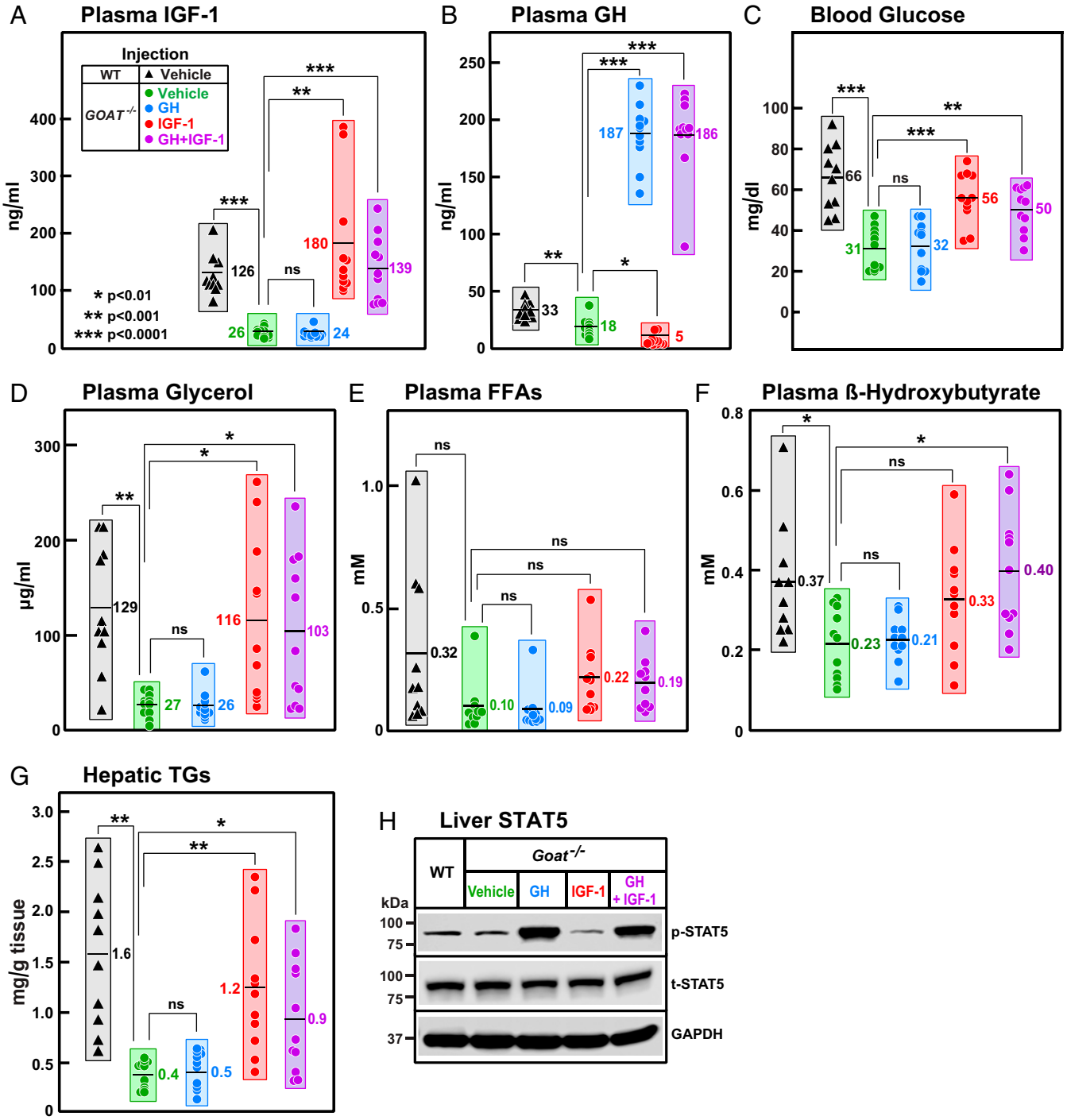

Fig. 2. IGF-1, but not GH, raises blood glucose in calorie-restricted *Goat*$^{-/-}$ mice and elicits differential metabolic responses. Male WT and *Goat*$^{-/-}$ littermates (8-wk old) were subjected to 60% calorie restriction for 6 d. On day 6 at 4:00 PM, mice were injected intraperitoneally with vehicle, GH (3 µg/g body weight), IGF-1 (100 ng/g body weight), or GH plus IGF-1. At 5:30 PM, blood was obtained for glucose measurements. At 6:00 PM, the mice were anesthetized, and blood and livers were obtained for measurements of the indicated plasma parameters (*A–F*), hepatic TGs (*G*), and immunoblot analysis (*H*), as described in Fig. 1. The data shown in *A–G* are from two independent experiments. Numbers beside the data points in *A–G* denote mean values. Asterisks (*) denote levels of statistical significance (Student's *t* test). ns denotes no significance; *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$. (*H*) Immunoblots of pooled aliquots of liver lysates from one of the two experiments were carried out as described in Fig. 1.

STAT5 was increased by injection of GH as well as GHRH. Thus, the failure of GH to raise IGF-1 was not due to a failure to activate STAT5 in the liver.

To determine whether the increase in plasma IGF-1 was responsible for the glucose-elevating effect of GHRH, we measured the response to injected IGF-1 (Fig. 2). Fig. 2*A* shows that plasma IGF-1 levels were severely reduced in calorie-restricted *Goat*$^{-/-}$ mice. To correct this deficit, we injected a dose of IGF-1 that raised plasma IGF-1 to levels that were similar to those in WT mice. Injection of GH did not raise plasma IGF-1, and it did not further raise the IGF-1 level when it was injected together with IGF-1. As shown in Fig. 2*B*, the *Goat*$^{-/-}$ mice had low GH levels, and they were raised by injection of GH, but not IGF-1. Fig. 2*C* shows the effects of these injections on blood glucose. As observed previously, blood glucose was low in *Goat*$^{-/-}$ mice. The level was not raised by injection

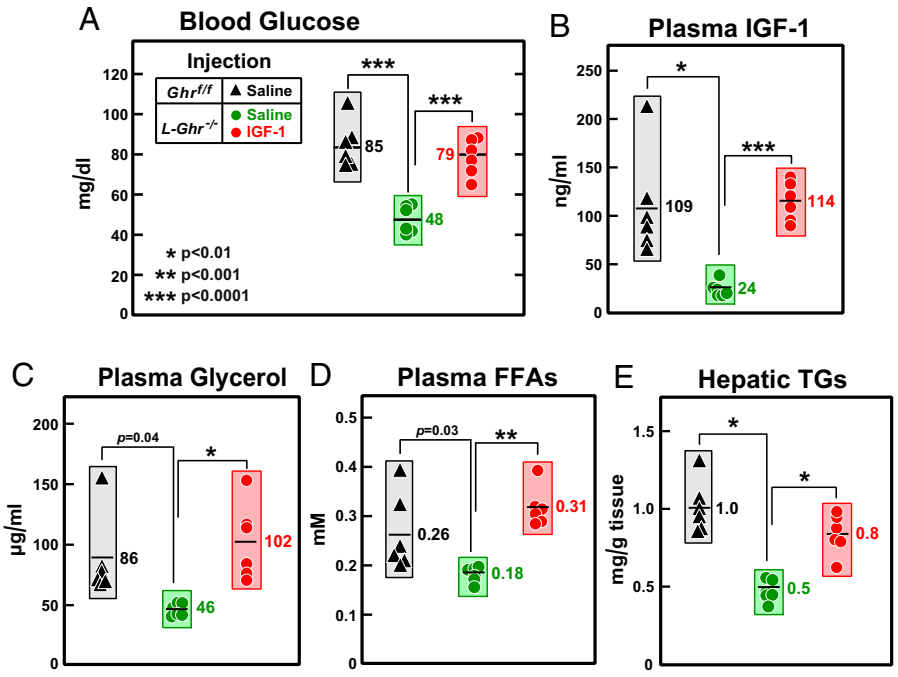

Fig. 3. Response of calorie-restricted *L-Ghr*$^{-/-}$ mice to injection of IGF-1. *L-Ghr*$^{-/-}$ male mice (8-wk old) and littermate male *Ghr*$^{f/f}$ controls (16) were subjected to 60% calorie restriction for 11 d. On day 11 at 4:00 PM, mice were injected intraperitoneally with saline or saline containing IGF-1 (100 ng/g body weight). (*A*) At 5:30 PM, blood was obtained for glucose measurements. (*B–E*) At 6:00 PM, the mice were anesthetized, and blood and livers were obtained for measurements of the indicated plasma parameters (*B–D*) and hepatic TGs (*E*), as described in Fig. 1. Numbers beside the plotted data denote mean values. Asterisks (*) denote levels of statistical significance (Student's *t* test). *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$.

of GH, but it was elevated by injection of IGF-1, and the addition of GH had no further effect.

We have shown previously that administration of energy sources, including a fatty acid (octanoate), pyruvate, or alanine can raise blood glucose in calorie-restricted *Goat*$^{-/-}$ mice (7). Fig. 2*D* shows that IGF-1, when given alone or with GH, raised one energy source: plasma glycerol. Plasma free fatty acids (FFAs) were also elevated, but the scatter in the data prevented the change from reaching statistical significance. To gain statistical significance, we pooled the data from the two groups of mice that received IGF-1 (with and without GH) and compared these values with the two groups that did not receive IGF-1 (with and without GH) (*SI Appendix*, Fig. S1*A*). The combined data confirmed that the IGF-1–treated animals had significantly elevated FFAs (0.2 mM vs. 0.1 mM, $P = 0.005$). Plasma β-hydroxybutyrate was also elevated in the mice that received IGF-1 (Fig. 2*E*), and statistical significance increased when the IGF-1–treated groups were pooled (*SI Appendix*, Fig. S1*B*). These findings indicate that the elevated plasma fatty acids are being taken up by the liver and oxidized to ketone bodies. The increased availability of fatty acids in the liver was further indicated by the observation that hepatic triglycerides (TGs) rose significantly when IGF-1 was injected (Fig. 2*G*). In the experiments of Fig. 2, as in the experiments of Fig. 1, GH increased phosphorylated STAT5 in the liver (Fig. 2*H*). In contrast, IGF-1 had no effect.

Mice that lack liver GH receptors (*L-Ghr*$^{-/-}$ mice) also develop hypoglycemia when subjected to 60% calorie restriction and a 23-h fast (16). The hypoglycemia is not as severe as seen in *Goat*$^{-/-}$ mice, and it takes longer to develop (11 vs. 6 d of calorie restriction). Fig. 3*A* shows that hypoglycemia can be alleviated by injection of IGF-1 in calorie-restricted *L-Ghr*$^{-/-}$ mice. After calorie restriction, the *L-Ghr*$^{-/-}$ mice had extremely low levels of plasma IGF-1 that were restored to WT levels by the IGF-1 injection (Fig. 3*B*). From a metabolic standpoint, the injected IGF-1 appeared to be acting in the same manner in the *L-Ghr*$^{-/-}$ mice as it did in *Goat*$^{-/-}$ mice. The peptide raised the plasma levels of glycerol and FFAs, and

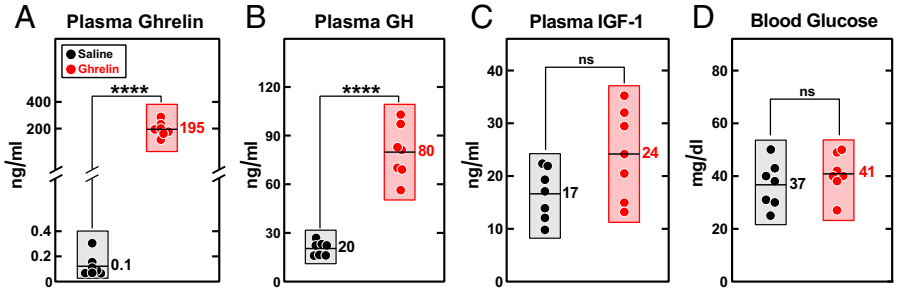

Fig. 4. Response of calorie-restricted *Goat*$^{-/-}$ mice to injection of ghrelin. Male *Goat*$^{-/-}$ mice (8-wk old) were subjected to 60% calorie restriction for 6 d. On day 6 at 4:00 PM, mice were injected intraperitoneally with saline or saline containing ghrelin (1 µg/g body weight). At 5:30 PM prior to anesthesia, blood was obtained for measurement of glucose (*D*). At 6:00 PM, the mice were anesthetized, and blood was obtained for measurement of plasma levels of ghrelin (*A*), GH (*B*), and IGF-1 (*C*), as described in *SI Appendix*. Numbers beside the plotted dots denote mean values. Asterisks (*) denote levels of statistical significance (Student's *t* test). ns, no significance; ****$P < 0.00001$.

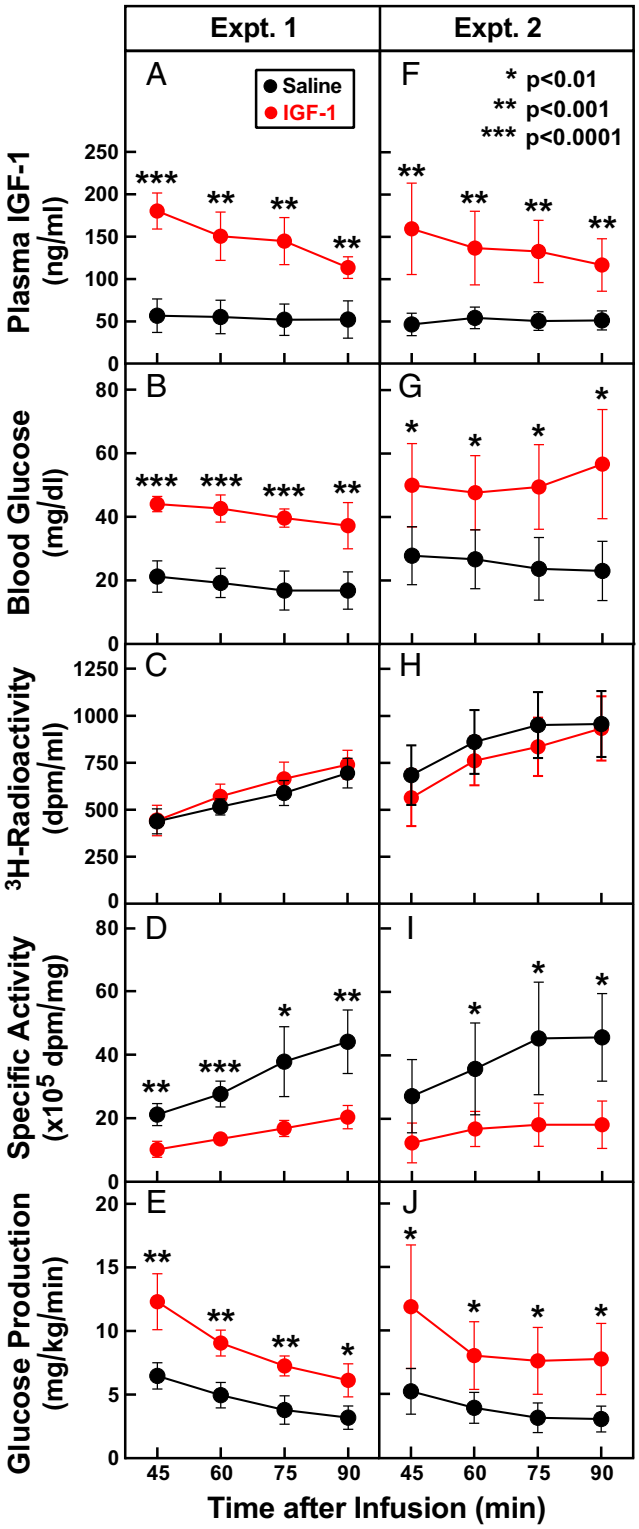

Fig. 5. Whole-body glucose production in calorie-restricted *Goat*$^{-/-}$ mice after IGF-1 injection. Data are presented for two independent experiments, designated Exp. 1 (*A*–*E*) and Exp. 2 (*F*–*J*). *Goat*$^{-/-}$ male mice (8-wk old) were implanted with jugular vein catheters 4 d before initiating 60% calorie restriction as described in *SI Appendix*. On day 6 at 4:00 PM, the mice were injected intraperitoneally with saline or saline containing IGF-1 (100 ng/g body weight), followed immediately by a continuous infusion of [3-^{3}H] glucose. The infusion rate was 0.3 μCi/min from 0 to 20 min, followed by 0.1 μCi/min from 20 to 90 min, after which blood was obtained at the indicated time for measurement of plasma IGF-1 (*A* and *F*), blood glucose (*B* and *G*), and ^{3}H-radioactivity in blood (*C* and *H*). Specific activity of blood glucose (*D* and *I*) and whole glucose production (*E* and *J*) were calculated as described in *SI Appendix*. Each point represents the mean ± SD of data from five (Exp. 1) or six (Exp. 2) mice. Asterisks (*) denote levels of statistical significance (Student's *t* test) between mice injected with IGF-1 and those injected with saline. *P < 0.01; **P < 0.001; ***P < 0.0001.

it increased hepatic TGs (Fig. 3 *C*–*E*). These data in mice that lack GH receptors further support the notion that the glucose-raising actions of IGF-1 are not dependent on GH action in the liver.

We have shown previously that hypoglycemia is prevented in *Goat*$^{-/-}$ mice when ghrelin is infused continuously through an osmotic minipump during the entire period of calorie restriction (1). Moreover, we found surprisingly that hypoglycemia was not reversed when ghrelin was injected acutely during the period of starvation following calorie restriction. One such experiment is shown in *SI Appendix*, Fig. S2. WT and *Goat*$^{-/-}$ mice were subjected to calorie restriction. Beginning on day 4 and continuing through day 8, we injected the mice intraperitoneally each day with ghrelin at 3:00 PM and again at 4:00 PM, and we measured the blood glucose at 5:30 PM. Blood glucose declined progressively from day 4 through day 8 in the *Goat*$^{-/-}$ mice, and the ghrelin injections had no effect.

Fig. 4 shows an experiment designed to determine whether the failure of acute ghrelin injection to raise blood glucose in calorie-restricted *Goat*$^{-/-}$ mice is due to a failure to raise IGF-1. We subjected *Goat*$^{-/-}$ mice to calorie restriction for 6 d. At 4:00 PM on day 6, we injected the mice with ghrelin or saline and measured the blood glucose at 5:30 PM. The ghrelin injection raised plasma ghrelin (Fig. 4*A*), and the ghrelin was active as indicated by a fourfold increase in plasma GH (Fig. 4*B*). However, plasma IGF-1 was not elevated significantly (Fig. 4*C*) and blood glucose failed to rise (Fig. 4*D*). Considered together with the other data in this paper, the result in Fig. 4 is consistent with the conclusion that relief of hypoglycemia requires an increase in plasma IGF-1, and that an increase in plasma GH, even when generated physiologically by an increase in ghrelin, cannot raise plasma IGF-1 or glucose.

To confirm that IGF-1 raises blood glucose in calorie-restricted *Goat*$^{-/-}$ mice by increasing glucose production rather than decreasing clearance, we used the same isotope dilution method that we used previously (7). Two similar experiments were performed (Exps. 1 and 2 in Fig. 5). Mice were injected with IGF-1 or saline and were then infused continuously with a solution of [^{3}H]glucose. Blood was withdrawn at the indicated times, and the specific radioactivity of glucose was determined. Increased glucose production led to lower specific radioactivity of glucose in the blood of animals that received IGF-1 (red symbols in Fig. 5 *D* and *I*) than in mice that received saline (black symbols in Fig. 5 *D* and *I*). Fig. 5 *E* and *J* show that the calculated glucose production rate was approximately twofold higher in the animals that received IGF-1. This explains the twofold higher blood glucose level in these mice (Fig. 5 *B* and *G*). It is noteworthy that blood glucose was maximally elevated as early as 45 min after IGF-1 injection, which was the first time point examined (Fig. 5 *B* and *G*).

Considered together, the data thus far raise the possibility that IGF-1 increases glucose production in calorie-restricted *Goat*$^{-/-}$ mice by mobilizing FFAs and glycerol and delivering them to the liver, where some of the fatty acids are oxidized to ketone bodies and some are stored as TGs. Fatty acid oxidation would supply energy, and glycerol would supply substrate for enhanced glucose production. Lipolysis in adipose tissue would be a logical source of the elevated plasma glycerol and fatty

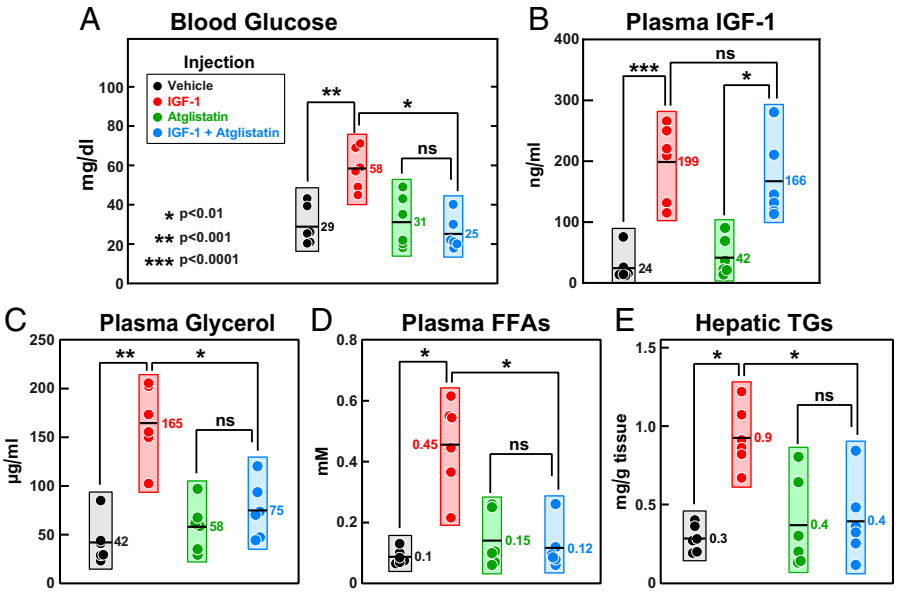

Fig. 6. Inhibition of ATGL blocks IGF-1–mediated increase in blood glucose in calorie-restricted *Goat*$^{-/-}$ mice. Male *Goat*$^{-/-}$ mice (8-wk old) were subjected to 60% calorie restriction for 6 d. On day 6 at 2:00 PM, mice were injected intraperitoneally with vehicle (20% DMSO/5% Cremophor in saline) or vehicle containing atglistatin (50 µg/g body weight). Two hours later at 4:00 PM, the mice were injected intraperitoneally with saline or saline containing IGF-1 (100 ng/g body weight). (*A*) At 5:30 PM, blood was obtained for glucose measurements. (*B–E*) At 6:00 PM, the mice were anesthetized, blood was obtained for measurement of the indicated plasma parameters (*B–D*), and sections of liver were obtained for measurement of hepatic TGs (*E*), as described in Fig. 1. Numbers beside the plotted points in *A–E* denote mean values. Asterisks (*) denote levels of statistical significance (Student's *t* test). *$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$.

acids. Even though calorie-restricted mice have a severe depletion of body fat (less than 2% of body weight as measured by NMR) (1), there might be enough residual fat to permit IGF-1–induced mobilization. To test this hypothesis, we used atglistatin, a potent inhibitor of adipose TG lipase (ATGL), the enzyme that initiates hydrolysis of TGs in white adipose tissue in mice (21).

As shown in Fig. 6*A*, after 23 h of fasting, blood glucose was low in calorie-restricted *Goat*$^{-/-}$ mice (mean value, 29 mg/dL). Injection of IGF-1 raised glucose by twofold to 58 mg/dL. This increase was blocked by prior administration of atglistatin. Fig. 6*B* shows that plasma levels of IGF-1 rose similarly in the injected animals in the absence and presence of atglistatin. As shown in Fig. 6 *C* and *D*, atiglistatin blocked the increases in plasma glycerol and FFAs in response to IGF-1, and also blocked the increase in hepatic TGs (Fig. 6*E*). These data are consistent with the suggestion that IGF-1 raises blood glucose by increasing lipolysis in the white adipose tissue of calorie-restricted mice.

Discussion

The present data reveal that ghrelin maintains viable levels of blood glucose in starved mice by preventing a profound decline in plasma levels of IGF-1. When ghrelin-deficient *Goat*$^{-/-}$ mice were subjected to calorie restriction followed by fasting, plasma IGF-1 levels were reduced by 80% when compared with similarly treated WT mice, and blood glucose was reduced by 50% (Figs. 1 and 2). Injection of IGF-1 doubled glucose production and doubled blood glucose levels within 45 min in the *Goat*$^{-/-}$ mice (Fig. 5). Increased glucose production was a consequence of increased lipolysis as indicated by increased plasma concentrations of glycerol and fatty acids (Fig. 2 and *SI Appendix*, Fig. S1). The glycerol and fatty acids were derived from lipolysis in white adipose tissue as indicated by the observation that the increase was blocked by administration of atglistatin, an inhibitor of ATGL (Fig. 6). The fatty acids were delivered to the liver as indicated by an increase in hepatic TGs (Fig. 2). Some of the hepatic fatty acids were oxidized to ketone bodies, as indicated by a rise in plasma β-hydroxybutyrate (Fig. 2), and this oxidation may have served as a source of ATP to support hepatic gluconeogenesis (7).

By revealing a hitherto unsuspected role for IGF-1 in starvation, the present data raise two important mechanistic questions: 1) What is the mechanism by which GHRH raises plasma IGF-1 in calorie-restricted mice? and 2) What is the mechanism by which IGF-1 stimulates lipolysis in adipose tissue of these animals?

With regard to the first question, GHRH action is generally thought to be confined to the pituitary, where it stimulates pulsatile secretion of GH (10, 12, 22, 23). Indeed, GHRH injection did raise plasma GH along with IGF-1 in our starved *Goat*$^{-/-}$ mice (Fig. 1), but the GH did not appear to be the cause of the increased IGF-1. This conclusion is based on the observation that direct injection of GH did not raise plasma IGF-1 even though it increased phosphorylation of STAT5 in the liver (Fig. 2). It is possible that the direct GH injection failed because it did not reproduce the physiologic pulsatile pattern of GH secretion that occurs in response to GHRH. However, as shown in Fig. 4, acute ghrelin injection raised plasma GH through a physiologic mechanism that is assumed to create pulsatile secretion (10). Yet, ghrelin injection did not raise plasma IGF-1 (Fig. 4).

Although GH is not able to raise plasma IGF-1 or glucose levels when injected acutely into starved *Goat*$^{-/-}$ mice, the hormone is required to prevent hypoglycemia during the chronic period of calorie restriction as indicated by the observation of hypoglycemia in calorie-restricted mice that lack GH receptors in the liver (Fig. 3). The paradox with GH is also seen with ghrelin. Chronically starved ghrelin-deficient mice become hypoglycemic, yet acute injection of ghrelin fails to increase blood glucose (Fig. 4). Considered together, these data indicate that during the long period of calorie restriction GH action in the liver is required to preserve IGF-1 levels and blood glucose. It is possible that ghrelin and GH must be present during the complete cycle of feeding and fasting in order to preserve blood glucose.

With regard to the second question, the most direct mechanism for IGF-1 stimulation of lipolysis would be a direct action on the IGF-1 receptors that are known to be present in white and brown adipose tissue (24). However, these receptors are thought to inhibit lipolysis rather than activating it (25, 26). It is also possible that IGF-1 stimulates lipolysis indirectly by reducing an inhibitor or increasing an activator of lipolysis. In this regard, pancreatic β-cells are known to express IGF-1 receptors (27, 28). β-Cells are the source of insulin, the major inhibitor of lipolysis. Is it possible that IGF-1 inhibits insulin secretion by pancreatic β-cells, and this removes an inhibitor of lipolysis? Against this hypothesis is the observation that plasma insulin levels are already barely detectable in our hypoglycemic calorie-restricted mice (1). The commercial assays for plasma insulin in mice are not sensitive enough to detect a fall from these already low levels if it occurs in response to IGF-1.

The observation that IGF-1 stimulates lipolysis and raises blood glucose in starved, ghrelin-deficient mice contrasts with the general observation that injected IGF-1 acts like insulin in reducing lipolysis (25, 26) and it lowers blood glucose in rodents and humans (29–31). A possible explanation lies in the low level of plasma IGF-1 that is required to stimulate lipolysis in starved mice. The plasma concentration of IGF-1 in starved WT mice (~120 ng/mL) is much lower than it is in WT mice that are not starved (~500 ng/mL) (32, 33). Blood glucose was restored in starved, ghrelin-deficient mice when we injected only enough IGF-1 to raise the plasma level to an average of 72 ng/mL (Fig. 1*C*). It is possible that high levels of IGF-1 are antilipolytic, while low levels are prolipolytic, particularly in the context of hypoglycemia.

Our observation that GH fails to stimulate IGF-1 production in starved *Goat*$^{-/-}$ mice is consistent with data in humans with anorexia nervosa. These subjects have high plasma GH but low plasma IGF-1 (34). This discrepancy has been interpreted to indicate hepatic resistance to GH in the anorexic state (34). The present data raise the possibility that the level of IGF-1 in anorexic subjects is kept in the low range where it stimulates lipolysis in order to maintain the blood glucose. This hypothesis is susceptible to testing in calorie-restricted mice and eventually in anorexic humans.

Materials and Methods

SI Appendix includes descriptions of the following items: reagents, mice; protocol for calorie restriction, solutions and injections, assays for various blood chemistries (glucose, hormones, glycerol, FFAs, β-hydroxybutyrate), measurement of liver TGs and whole-body glucose production, and immunoblot analysis.

Mice. *Goat*$^{-/-}$ mice were generated as previously described (1). *L-Ghr*$^{-/-}$ and *Ghr*$^{f/f}$ mice were generated as previously described (16). All animal experiments were approved and conducted under oversight of the University of Texas Southwestern Institutional Animal Care and Use Committee.

Reproducibility. All experiments were repeated multiple times on different days. Similar results were obtained.

Data Availability. All study data are included in the main text and *SI Appendix*.

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