Glucagon-initiated human growth hormone release: a comparative study

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Summary: Human growth hormone (HGH) responses in 20 healthy adults to subcutaneous glucagon, arginine infusion and tolbutamide and insulin hypoglycemia were compared. HGH rose in all four tests. HGH response to glucagon was also studied in 49 patients with suspected pituitary insufficiency, of whom 25 also later received an arginine infusion; an abnormal response to glucagon was the most frequent functional abnormality and often HGH was the only anterior pituitary hormone of which a deficiency was detectable. In seven subjects (two healthy controls and five patients with suspected hypopituitarism) there was a subnormal HGH response to arginine but a normal response to glucagon. It is concluded that glucagon is a simple and effective stimulus to HGH release, equal or superior to arginine, tolbutamide and insulin, and is an important test of anterior pituitary function.

Résumé: ***Libération de l'hormone somatotrope humaine, induite par le glucagon: étude comparative***

Les chercheurs ont comparé chez 20 adultes sains la réaction de la somatotrophine (ST) à divers stimuli: glucagon sous-cutané, perfusion d'arginine et hypoglycémie tolbutamidique et insulinique. Ces quatre essais ont eu pour résultat d'augmenter la libération de ST. La réaction de la ST au glucagon a été également étudiée chez 49 malades soupçonnés d'insuffisance hypophysaire, 25 d'entre eux ayant reçu ultérieurement une perfusion d'arginine. Une réaction anormale au glucagon a été l'anomalie fonctionnelle la plus fréquente et, fréquemment, la ST était la seule insuffisance de l'antéhypophyse qui ait pu être décelée. Chez sept sujets (deux témoins sains et cinq malades soupçonnés d'hypopituitarisme), on notait une réaction subnormale de la ST à l'arginine, mais une réaction normale au glucagon. Les auteurs concluent que le glucagon est un stimulus simple et efficace à la libération de ST, égal ou supérieur à l'arginine, au tolbutamide et à l'insuline et qu'il constitue une épreuve capitale de la fonction de l'antéhypophyse.

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Evaluation of pituitary growth hormone release is important in the general assessment of anterior pituitary function. Many stimulatory tests have been employed in determining human growth hormone (HGH) release as single fasting samples have failed to differentiate clearly between the normal and the HGH-deficient individual.[1] The two most widely employed stimuli are hypoglycemia (induced by either insulin or tolbutamide) and the arginine infusion test.[2-4] Because of side-effects and technical difficulties[5] the clinical use of these tests has not been entirely satisfactory and attempts have been made to develop improved methods.

Mitchell *et al*[6] have reported that glucagon is a reliable, simple and innocuous test of HGH release. However since 1963, when the first report of HGH response to glucagon appeared, there have been conflicting claims about its effectiveness.[7-12] In a previous report this laboratory confirmed that subcutaneous glucagon stimulates HGH release, and presented evidence that the reported discrepancies in HGH response to glucagon might be methodological.[13] No comparative study of the HGH response to glucagon and to other standard stimuli in the same subjects, measured by the same assay system, has been described. Similarly, since the report by Mitchell *et al*,[6] no detailed account of the use of the glucagon test in hypopituitarism has been published. The present study compares the HGH response to glucagon, arginine and hypoglycemia in normal man with the response to glucagon in 49 patients with suspected anterior pituitary dysfunction.

Methods

The control (normal) subjects were 20 non-obese healthy volunteers, 14 males and six females, aged 21 to 34 years, who were hospitalized in The Clinical Research Center of the Peter Bent Brigham Hospital and fed a constant isocaloric diet. Their medical history and physical examination gave no evidence of recent clinical disease. All had normal serum thyroxin, resin thyroxin uptake and 24-hour urine total gonadotrophins, 17-ketosteroids and 17-hydroxycorticosteroids. Fasting plasma glucose and

an oral or intravenous glucose tolerance test were normal in each subject and none had a family history of diabetes mellitus.

Each subject was tested with both an arginine infusion and glucagon. Ten subjects were also tested with insulin- and tolbutamide-induced hypoglycemia. A minimum of 48 hours elapsed between each test. The arginine was given as a 5% levoarginine monochloride infusion* (30 g. over one-half hour). The dose of arginine ranged from 0.17 to 0.33 g./lb. body weight. The males received oral diethylstilbestrol, 2.5 mg. twice on the day before and 2.5 mg. at 7 a.m. on the arginine test day. Glucagon** was given as a 1 mg. subcutaneous injection. Insulin was given as crystalline zinc insulin, 0.05 U./kg. body weight in an IV bolus. In the tolbutamide test 1.0 g. of tolbutamide dissolved in 20 ml. of normal saline was given IV over two minutes. The tests were started between 8:00 and 9:00 a.m. after an overnight fast and before ambulation. The subjects were supine throughout each test. Thirty minutes prior to the start of the test a 19-gauge scalp vein needle was introduced into an antecubital vein and was used for subsequent sampling through a double stopcock.[14]

Forty-nine patients with clinically suspected or diagnosed hypopituitarism were studied in the hospital. They ranged in age from 21 to 75 years. In many cases the patients were already receiving either thyroid or adrenal replacement therapy; this was continued during the study but all other medications were discontinued. Serum thyroxin, resin thyroxin uptake, 24-hour urinary total gonadotrophins, 17-ketosteroids (17-KS), 17-hydroxycorticosteroids (17-OH), and urinary hydroxycorticosteroid and tetrahydrodeoxycortisol response to oral metyrapone (750 mg. q4h over 24 to 48 hrs.) were measured in most patients. The normal ranges for these are given in Table III. Twenty-four-hour urinary total gonadotrophin excretion was interpreted in conjunction with age and evidence of gonadal function (levels of serum testosterone and urinary estrogen excretion or vaginal cytology). Where appropriate, in order to exclude primary glandular failure adrenocorticotrophin, human chorionic gonadotrophin and thyrotrophin tests were performed. Each patient was tested with a glucagon infusion and 25 patients also received an arginine infusion. In eight of the patients who failed to demonstrate an HGH response to glucagon the test was repeated.

Plasma glucose was determined by the ferrocyanide procedure described by Hoffman[15] and modified for use with the AutoAnalyzer. Plasma cortisol was determined by the competitive protein-binding method of Nugent and Mayes[16] and free fatty acids (FFA) by the method of Dole and Meinertz.[17] HGH was measured by a double antibody radioimmunoassay.[3] All values are reported as means plus or minus the standard error of the mean (SE) unless otherwise specified. Statistical calculations were performed by Student's t test and correlation coefficients with the aid of a General Electric 635 computer.[18]

*Cutter Laboratories, Berkeley, California.
**Crystalline Glucagon, Eli Lilly & Co., Indianapolis.

Results

Control (healthy) subjects

Glucagon testing: The 1 mg. s.c. glucagon injection produced a significant ($P<0.001$) mean HGH rise to 21.8 ± 3.9 ng./ml. at 150 mins. (Table I). The mean of the individual maximum HGH responses was 24.1 ± 4.1 ng./ml. and the range of the individual maximum HGH increases above base line was 2.3 to 74.4 ng./ml. Only one of the subjects showed a blunted peak HGH response to glucagon of under 5 ng./ml. (peak response 2.8 ng./ml.; rise above base line 2.3 ng./ml.). He was a 27-year-old male of normal stature and growth history whose other metabolic responses to glucagon were similar to those of the other test subjects except for failure to show an HGH rise. No significant side-effects were noted except that two subjects complained of nausea at 90 to 120 mins. In general there was no difference in the male and female HGH responses although the basal levels and the maximum responses tended to be higher in the six female subjects.

The plasma glucose response was characterized by an initial rise followed by a slow fall to below basal levels. The plasma glucose nadir at 120 mins. was 18.9 ± 1.9 mg./100 ml. below basal levels. There was a significant ($P<0.05$) negative correlation between the blood sugar nadir at 120 mins. and the maximum HGH response ($r = -0.447$). The plasma cortisol fell from 13.5 ± 1.0 μg./100 ml. to 11.8 ± 1.4 μg./100 ml. at 60 mins. but

TABLE I

MEAN GLUCOSE, CORTISOL, HGH, AND FFA RESPONSE TO 1 MG. SUBCUTANEOUS GLUCAGON IN TWENTY NORMAL SUBJECTS

	Time (minutes)											
	-30	0	15	30	45	60	90	120	150	180	210	240
Plasma Glucose mg./100 ml. ± SEM	94 ±1.7	93 ±1.8	125 ±5.2	159 ±6.4	149 ±7.5	135 ±9.0	92 ±4.8	76 ±2.9	76 ±1.8	78 ±1.6	82 ±1.6	85 ±1.8
Plasma Cortisol μgm/100 ml. ± SEM	13.5 ±1.0	---	---	12.8 ±1.5	---	11.8 ±1.4	---	12.4 ±1.3	---	15.8 ±1.3	---	13.2 ±1.6
Serum HGH ng./ml. ± SEM	0.8 ±0.2	0.7 ±0.1	0.6 ±0.1	0.6 ±0.1	0.6 ±0.1	0.7 ±0.1	3.0 ±0.7	11.9 ±2.3	21.8 ±3.9	15.4 ±3.5	7.0 ±1.5	4.1 ±0.9
Serum FFA mEq./L. ± SEM	0.38 ±0.16	---	---	0.31 ±0.12	---	0.22 ±0.05	---	0.23 ±0.05	---	0.62 ±0.2	---	0.86 ±0.4

then rose significantly ($P<0.05$) to a peak of 15.8 ± 1.3 μg./100 ml. at 180 mins. The serum FFA initially fell from 0.38 ± 0.16 mEq./l. (basal) to 0.22 ± 0.05 mEq./l. (60 mins.) and 0.23 ± 0.05 mEq./l. (90 mins.). The serum FFA then rose significantly ($P<0.05$) to 0.86 ± 0.4 mEq./l. (240 mins.). There was a significant ($P<0.05$) correlation between the late FFA rise and the preceding HGH peak ($r = 0.469$).

Arginine infusion: In response to a 30 g. arginine infusion the serum HGH rose significantly ($P<0.001$) to 14.5 ± 0.6 ng./ml. at 60 mins. (Table II). Three males however failed to show a significant serum HGH rise (peak response of 2.6, 2.7 and 2.4 ng./ml.; rise above base line 2.2, 2.1 and 2.3 ng./ml. respectively); one had not shown a normal HGH response to glucagon while the other two had responded normally. The dosage of arginine in the three non-responders was 0.18, 0.21 and 0.25 g./lb. body weight. No significant side-effects were noted.

Tolbutamide and insulin hypoglycemia: The ten subjects who were tested with insulin and tolbutamide hypoglycemia all showed significant ($P<0.01$) HGH rises to 21.5 ± 5.2 ng./ml. and 10.5 ± 1.0 ng./ml., respectively (Table II). The one male who had not shown a significant HGH response to glucagon and arginine was not tested with either insulin or tolbutamide hypoglycemia; the other two controls who showed a blunted response to arginine responded normally to insulin and tolbutamide hypoglycemia. The plasma glucose nadir of 33.0 ± 3.0 mg./100 ml. at 30 mins. in the insulin tolerance test was significantly ($P<0.01$) below basal levels and all subjects experienced hypoglycemic symptoms. The degree of hypoglycemia in the tolbutamide test was less pronounced (50 ± 2 mg./100 ml. at 40 mins.) and symptoms were not as severe.

Patients with suspected hypopituitarism

Both an arginine and a glucagon test were performed in 25 patients and the HGH responses to each stimulus compared. The HGH rise of 3.4 ± 0.9 ng./ml. in response to glucagon was significantly greater than to arginine (1.7 ± 0.5 ng./ml.). Of more importance however was the fact that five patients (1, 2, 12, 13 and 46) who showed a subnormal response to arginine (under 5 ng./ml. rise) and therefore might have been considered to have pituitary HGH insufficiency, showed a normal HGH response to glucagon. Only two patients who had a subnormal HGH response to glucagon showed a normal HGH response to arginine.

Of the 49 patients who were tested 16 were found to have apparently normal anterior pituitary function although five (12 to 16) had radiological evidence of sella turcica enlargement or erosion. Patients 7, 8 and 11 were considered to have primary hypothyroidism based on the finding of low serum thyroxin levels, low resin thyroxin uptake and high titres of thyroid antibodies

TABLE II

PLASMA GLUCOSE AND HGH RESPONSE IN NORMAL SUBJECTS TO ARGININE, TOLBUTAMIDE AND INSULIN

Stimulus	Time (minutes)											
	0	10	20	30	40	45	50	60	90	120	150	180
Arginine Infusion* (n = 20)												
Mean Plasma Glucose (mg./100 ml. ± SEM)	88 ±1	103 ±2	105 ±3	105 ±4	--	95 ±3	--	81 ±2	80 ±2	87 ±2	92 ±2	94 ±2
Mean Serum HGH (ng./ml. ± SEM)	1.4 ±0.4	2.2 ±0.5	4.2 ±0.9	5.4 ±1.1	--	11.4 ±2.9	--	14.5 ±0.6	10.6 ±2.1	6.7 ±1.2	4.3 ±0.7	2.3 ±0.6
Insulin Hypoglycemia† (n = 10)												
Mean Plasma Glucose (mg./100 ml. ± SEM)	92 ±2	63 ±5	45 ±4	33 ±3	46 ±3	--	53 ±4	66 ±4	68 ±4	77 ±3	85 ±3	84 ±2
Mean HGH (ng./ml. ± SEM)	1.2 ±0.3	1.3 ±0.4	1.8 ±0.4	4.3 ±1.6	7.7 ±2.1	--	20.0 ±4.9	21.5 ±5.2	19.5 ±7.7	13.9 ±4.9	9.5 ±3.5	6.5 ±2.6
Tolbutamide Hypoglycemia‡ (n = 10)												
Mean Plasma Glucose (mg./100 ml. ± SEM)	89 ±2	71 ±2	59 ±4	51 ±3	50 ±2	--	50 ±1	56 ±4	61 ±2	70 ±2	73 ±1	79 ±1
Mean HGH (ng./ml. ± SEM)	0.8 ±0.2	0.9 ±.2	0.8 ±.1	1.1 ±0.3	3.7 ±0.9	--	4.5 ±1.4	8.7 ±2.5	10.5 ±1.0	6.4 ±1.9	3.4 ±0.9	2.2 ±0.5

*30 gm. intravenously over 1/2 hr.
†intravenous bolus - 0.05 U/Kg body weight
‡1.0 gm. intravenously over 2 minutes

either at the time of study or prior to starting thyroid replacement therapy.

In 33 patients varying degrees of anterior pituitary insufficiency were present although it was often difficult to ascertain the degree of dysfunction. It was particularly difficult to interpret urinary total gonadotrophin findings since the range of normal varies widely according to sex, age and degree of end-organ function. The most frequent functional disturbance was HGH deficiency (28 patients) while urinary total gonadotrophins were abnormally low in only 20 patients and deficiencies of either ACTH or TSH were much less frequent. It is important to note that in six patients (28-33) the only functional abnormality detected was HGH deficiency

TABLE III

ENDOCRINE PROFILE IN FORTY-NINE PATIENTS WITH SUSPECTED HYPOPITUITARISM

Patient	Age (yr.)	Sex	Serum Thyroxin (µg/100 ml.)	Urine 17-OH (mg/day)	Urine 17-KS (mg/day)	Urine 17-OH Increase to Metyrapone* (%)	Urine Gonadotropins (U/day)	Sella Size**	Medications***	Maximum HGH Rise Over Base Line to Arginine (ng/ml.)	Glucagon (ng/ml.)
Normal Value			3.5-11	2-10	5-15 (F) 10-20 (M)	>60	10-200 (F)**** 10-50 (M)			5 ng.	5 ng.
Group A: No Pituitary Insufficiency:											
1	69	F	4.2	6.7	4.4	---	100	0	0	1.0	5.4
2	23	M	4.3	3.4	5.1	---	---	0	0	1.9	6.7 (5.2)†
3	62	F	4.9	8.5	6.4	---	20	0	0	---	13.0
4	51	F	5.8	6.3	3.7	---	20	0	0	---	19.0
5	65	F	3.5	6.7	5.5	200	100	-	0	---	19.6
6	56	F	7.4	8.9	7.8	---	200	0	0	---	19.0
7	23	F	2.8	5.6	10.8	---	10	0	0	---	12.3
8	40	F	8.2	3.9	3.9	---	50	0	T	---	22.0
9	33	F	3.2	5.2	4.7	80	20	0	0	5.4	13.3
10	55	M	5.8	8.8	12.5	---	10	0	0	---	2.0 (4.4)†
11	38	F	4.6	2.6	2.2	---	20	0	T	4.8	11.8
12	68	F	5.3	8.0	3.9	80	50	E	0	1.5	9.2
13	50	F	6.1	7.0	6.0	75	100	E	0	0	5.2
14	62	M	5.2	4.2	3.8	80	20	E	0	---	8.0
15	59	M	4.6	6.6	8.6	90	10	E	0	---	15.0
16	68	F	7.8	5.2	8.5	60	100	E	0	---	35.5
Group B: HGH Deficiency:											
17	30	F	3.5	---	---	---	0	E	CT	0.3	2.6
18	45	M	2.9	1.2	3.6	0	0	0	0	0.4	0.3
19	26	M	5.6	---	---	---	0	E	CT	0	1.4
20	31	F	7.1	---	---	---	0	E	CT	0	0
21	42	M	4.2	2.2	1.0	0	0	0	T	0	0
22	42	F	1.0	2.5	2.1	---	0	E	0	---	0
23	31	M	7.8	---	---	---	0	E	CT	---	2.9 (0.3)†
24	75	F	1.3	---	---	---	5	E	CT	---	0.9
25	29	M	5.4	---	---	---	0	E	CT	1.2	0.9
26	64	M	2.4	2.0	3.1	0	0	E	0	0.9	1.3
27	25	F	2.2	1.7	3.7	15	10	0	T	0	0
28	33	M	4.3	---	---	---	20	E	C	1.1	0.5
29	59	F	4.0	8.1	4.6	240	100	E	0	4.1	2.3
30	46	F	4.8	6.2	8.8	---	10	E	0	---	3.5
31	49	M	5.5	7.8	12.0	250	10	E	0	---	0 (0)†
32	54	M	6.4	2.4	3.2	80	10	E	0	1.0	1.0
33	34	M	5.4	10.7	10.0	60	50	E	0	---	0.3
34	23	M	4.2	4.8	8.5	0	20	E	0	0.8	0.1
35	24	M	4.9	4.2	5.1	20	5	0	0	0	0 (0)†
36	21	M	6.0	6.9	4.5	80	0	0	0	4.6	2.1
37	51	F	3.7	8.5	6.5	175	10	E	T	0	0
38	32	M	4.5	5.4	9.6	120	0	0	0	0	0
39	35	F	4.4	3.7	4.6	100	0	0	T	4.7	2.5
40	41	F	5.7	---	---	---	0	0	CT	---	0.5 (0)†
41	32	F	5.9	11.1	4.6	20	10	E	T	---	3.2
42	27	F	3.6	5.7	9.5	---	5	0	T	---	2.8
43	54	M	3.7	10.0	9.3	0	0	E	0	---	0
44	50	F	4.6	0.9	1.9	---	0	0	0	---	1.6
Group C: Pituitary Insufficiency other than HGH											
45	33	F	0.7	12.1	14.9	0	20	E	0	---	13.3
46	56	M	4.9	3.1	3.1	50	100	E	T	0.2	5.5
47	28	F	5.6	5.6	10.0	0	50	0	0	9.7	13.0
48	56	F	5.6	1.8	2.9	---	50	E	0	---	7.3
49	49	F	2.1	4.6	5.7	---	0	0	0	---	11.6

* 750 mg. p.o. q4h over 24 to 48 hours
** 0 = no enlargement; E = enlargement
*** C = cortisone or equivalent; T = thyroid replacement medication; 0 = none
**** Normal value depends on age and degree of ovarian or testicular function
† Repeat glucagon test

although each had evidence of sella turcica enlargement. Patient 28 had undergone bilateral adrenalectomy for treatment of Cushing's syndrome three years before and was on full cortisone replacement when studied. He was not considered to have an ACTH deficiency since he was suffering from Nelson's syndrome, characterized by increased skin pigmentation which subsided after surgical hypophysectomy.

Discussion

Impairment of pituitary HGH release is frequently the only hormone deficiency in hypopituitarism.[1] Our study of 49 patients with suspected anterior pituitary insufficiency and/or sella enlargement confirms that not only is the reduction in HGH release frequently the only detectable functional pituitary deficiency but also that it is the most frequently encountered defect. Therefore assessment of pituitary function should include appropriate stimulatory testing for HGH release.

The standard tests however has significant side-effects. Hypoglycemia, although it consistently produces HGH rises in normal subjects, may be severe and prolonged in patients with pituitary, adrenal or hepatic insufficiency and therefore requires the attendance of a physician. The standard arginine infusion test also has disadvantages in that it requires pretreatment of male patients with estrogens and also a half-hour intravenous infusion of approximately 600 ml. of fluid in order to reach the maximal effect. The glucagon test is less complicated in that it can be given as a subcutaneous injection and does not require the attendance of a physician. The only relative contraindications to the glucagon test are suspected pheochromocytoma and insulinoma.

Recent reports have questioned the reliability of glucagon in stimulating a growth hormone response in normal man.[10, 11] It is difficult to compare reported HGH responses to various stimuli unless the responses to various stimuli are studied in the same subjects and measured by the same assay. For our control subjects glucagon was a potent stimulus to HGH release. In both the patients with suspected hypopituitarism and the controls the mean HGH rise after glucagon injection was greater than that after arginine infusion. Also the percentage of patients and controls showing a normal HGH rise was greater with glucagon than with arginine. Similarly in normal subjects both the magnitude of the HGH response and the percentage of responders to glucagon were equal to or greater than those produced by insulin or tolbutamide testing.

The mechanism of glucagon-induced HGH release is obscure. It seems probable that glucagon induces HGH release by indirect means although a direct mechanism is not excluded. It seems likely that the relative hypoglycemia at 90 to 150 mins. after the glucagon injection is the stimulus to HGH release. The positive correlation between the HGH rise and the degree of hypoglycemia induced, and the observation by Eddy, Jones and Hirsch,[11] that hyperglycemia can obliterate the HGH response to glucagon support this concept. However the magnitude of the HGH rise is greater than would be expected with the relatively low degree of hypoglycemia. In glucose, tolbutamide and insulin tolerance tests the degree of hypoglycemia needed to stimulate an equivalent HGH rise is much greater.[2, 3, 19, 20] It would appear that an additional factor(s) augments the HGH response.

Recently it has been shown that the HGH response to arginine infusion may be abolished or depressed by elevating the FFA concentration.[21] Irie *et al*[22] have demonstrated that lowering of FFA with nicotinic acid causes a rise in HGH 120 to 180 mins. after initiation of the infusion. They have been able to obliterate this HGH response with concomitant heparin administration and the maintenance of a constant FFA level. In our study although the basal FFA levels tended to be low there was a consistent early FFA decrease as the lipolytic effects of glucagon were counteracted by the large insulin response. It is therefore probable that the low fatty acid level augments the HGH response to glucagon. Further studies in which FFA levels are maintained at a normal or elevated level will be necessary to clarify their role in the HGH response to glucagon.

Because the HGH response to various stimuli does not appear to be an all-or-none phenomenon it is difficult to define a normal response to glucagon. Based on the responses of the subjects in this and other studies[13] an HGH rise of less than 5 ng./ml. would appear to be abnormal and suggestive of pituitary insufficiency. As in all tests for HGH release, including glucagon, control subjects occasionally fail to demonstrate an appropriate HGH rise when stimulated. When this occurs in testing patients a repeat glucagon test and, if warranted, an arginine, insulin or tolbutamide test should be done before concluding that the patient has definite HGH deficiency.

In clinical practice, sampling times during the glucagon test may be reduced to 0, 120, 150, 180 and 210 minutes after injection without missing the peak HGH response. The finding of a normal HGH response is against the diagnosis of hypopituitarism but does not exclude the possibility that other isolated deficiencies may exist. Conversely the finding of a subnormal HGH response to glucagon, especially if confirmed by a repeat test, is indicative of pituitary insufficiency and a complete pituitary assessment is indicated.

In summary, the glucagon test is a simple, potent, reliable and innocuous stimulus to HGH release in both healthy subjects and patients with pituitary dysfunction or insufficiency. In normal man it is as potent as insulin-induced hypoglycemia and more potent than arginine or tolbutamide-induced hypoglycemia. In patients with pituitary dysfunction glucagon is also effective in detecting HGH deficiency, often the first evidence of pituitary insufficiency. The probable mechanism of the glucagon-induced HGH release is a fall in plasma glucose acting in conjunction with a reduced FFA level.

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