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Effects of Starvation on *in Vivo* Gluconeogenesis in Hypo- and Hyperthyroid Rats*

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ABSTRACT. Studies were performed on rats *in vivo* to determine whether starvation disrupts glucose metabolic balance after removal of the thyroid gland. Intact controls and thyroidectomized rats were injected daily with 0, 0.1, 1.8, or 25 μg L-T_4/100 g BW. Glucose spaces were similar in all groups. The disappearance of labeled glucose from blood was faster in the thyroidectomized rats injected with 25 μg L-T_4 than in the other groups. Starvation enhanced the production of [^{14}C]glucose from [3-^{14}C]pyruvate in all groups, but this effect occurred earlier in control rats and thyroidectomized rats given 1.8 or 25 μg L-T_4 than in thyroidectomized rats given either 0 or 0.1 μg L-T_4. Starvation also enhanced the appearance of radioactivity in liver glycogen 30 min after the injection of [3-^{14}C]pyruvate in all groups, but this effect was lesser in thyroidectomized rats given 0, 0.1, or 25 μg L-T_4 than in other groups. The normal net production of glucose in fed thyroidectomized rats may be the result of a balanced equilibrium between reduced gluconeogenesis and glycolysis. Results obtained in thyroidectomized rats given 25 μg L-T_4 are discussed in terms of the augmented utilization of the newly formed glucose which compensates for their enhanced gluconeogenesis. (*Endocrinology* **106**: 1628, 1980)

THE EFFECTS of changes in thyroid function on the activity of gluconeogenic enzymes in the liver are controversial (1–11). While the rate of gluconeogenesis in hypo- and hyperthyroid rats has been studied in the liver *in vitro* (12–14), *in vivo* studies have not been reported. Food deprivation disrupted metabolic balance in the liver and blood of both hypo- and hyperthyroid animals (15, 16). The present work was carried out to relate previous findings to an index of *in vivo* gluconeogenesis, the appearance of [^{14}C]glucose in the blood after [3-^{14}C]pyruvate administration to fed and starved thyroidectomized animals injected daily with L-T_4. In addition, the disappearance rate of [*U*-^{14}C]glucose from blood was studied in order to determine the glucose space and glucose half-life in fed animals with different thyroidal states.

Materials and Methods

Animals

Young male Wistar rats, weighing 40–50 g, were fed a medium residue, low iodine diet of the Remington type (17). They were surgically thyroidectomized (18) and then injected ip daily with 0, 0.1, 1.8, or 25 μg L-T_4/100 g BW for 40 days. Age- and sex-matched intact controls received the same diet supplemented with 1.7 μg $KClO_3$/g and a daily injection of 0.9% NaCl. Animals were housed in an air-conditioned room maintained at 22–24 C with a 12-h light, 12-h dark cycle. All experiments were performed at the onset of the light cycle, and starvation was induced by removal of food at preset times of darkness to avoid differences in food intake during the day. The animals received distilled water *ad libitum*.

Gluconeogenesis from labeled pyruvate in vivo

Unanesthetized rats were injected ip with 0.5 ml/200 g BW of a solution containing 1×10^7 dpm [3-^{14}C]pyruvate (SA, 8.3 mCi/mmol; Radiochemical Center, Amersham, England) supplemented with 0.5 mmol sodium pyruvate to minimize artifacts due to the different weights of the animals as well as variations in the dilution of the tracer by endogenous metabolites. Blood was collected 5 and 15 min later, dropwise, from the cut tip of the tail into heparinized plates. Animals were decapitated 30 min after the injection of [3-^{14}C]pyruvate, and blood was collected from the neck. Sections of liver were immediately frozen in liquid N_2 for subsequent isolation of labeled glycogen.

To analyze [^{14}C]glucose in whole blood, aliquots were deproteinized with $Ba(OH)_2$-$ZnSO_4$ (19), and aliquots of protein-free supernatants were passed over microcolumns of Dowex 1 × 2-400-activated Duolite A-4 (OH)-Dowex 1 × 2-400, prepared as previously described (20). The columns were rinsed with 3 ml deionized distilled water, and the eluates were counted and analyzed for glucose with glucose oxidase (21). Recovery of [^{14}C]glucose added to blood before precipitation was 99.2–102%

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by this technique, whereas the recoveries of added [^{14}C]alanine, [^{14}C]lactate, and [^{14}C]pyruvate were less than 0.15%, 0.09%, and 0.25%, respectively.

Glycogen was precipitated after alkaline digestion (22) of aliquots of the frozen liver. The glycogen was resolubilized in water and reprecipitated twice with ethanol for purification before acid hydrolysis (2.5 M H_2SO_4 for 1 h at 100 C). Aliquots of the acid hydrolysates were counted and analyzed for glucose with glucose oxidase (21). This procedure was previously validated by chromatographic methods (20).

Disappearance of glucose in vivo

The rats were anesthetized with ether-O_2/CO_2 (95-5) mixture, and the femoral vein and carotid artery were cannulated with a polyethylene tube (0.59 mm od/0.57 mm id). Animals were injected via the femoral vein with 0.4 ml/200 g BW of a solution containing 1×10^7 dpm [*U*-^{14}C]glucose (SA, 3 mCi/mmol; Radiochemical Center). Blood was collected from the cannula placed in the carotid artery 1, 2, 4, 6, 8, 10, 20, and 30 min after injection to measure [*U*-^{14}C]glucose, as described above.

Radioactive assay

The composition of the liquid scintillation counting medium used was: 3 g 2.5-diphenyloxazole (PPO), 100 mg 1,4-bis [2-(5-phenyloxazolyl)]benzene (POPOP), 750 ml xylene, and 750 ml Triton X-100. Counting of radioactivity was done in a Nuclear-Chicago Isocab 300 scintillation counter fitted with an external standard device (Nuclear-Chicago, Des Plaines, IL).

Expression of results

The glucose space was calculated by the method of Baker *et al.* (23). The time at which the radioactivity of glucose in blood was half the value of that at zero time was considered the half-life of the injected glucose. The radioactivity of administered [3-^{14}C]pyruvate converted to labeled glucose at various intervals after injection was calculated as: labeled glucose (disintegrations per min/100 g BW) = glucose space (milliliters per 100 g BW) × disintegrations per min of [^{14}C]glucose/ml blood. The incorporation of labeled pyruvate into hepatic glycogen was calculated as: labeled glycogen (disintegrations per min/100 g BW) = disintegrations per min of [^{14}C]glycogen/g liver × liver wt (grams) × 100/g BW. The specific radioactivity for glucose in blood and liver glycogen was expressed as disintegrations per min/mg.

Results

Body weight, liver weight, and body size

Body and liver weights and body size (Table 1) were significantly lower in thyroidectomized rats treated with either 0 or 0.1 μg T_4 than in the intact control rats. There were no differences between thyroidectomized rats given 1.8 μg T_4 and control rats in this respect, while thyroidectomized rats treated with 25 μg T_4 showed a significant reduction in body weight.

TABLE 1. Effect of thyroidectomy and treatment with L-T_4 on body and liver weights and body size in the rat

Group (μg L-T_4/100 g BW)[a]	BW (g)	Body size (cm)[b]	Liver wt (g)
Intact controls (0)	192.9 ± 4.1 (70)[c]	19.6 ± 0.3 (10)	10.64 ± 0.35 (17)
Thyroidectomized (0)	103.2 ± 6.7 (80)	14.9 ± 0.4 (10)	4.64 ± 0.68 (10)
P[d]	<0.001	<0.001	<0.001
Thyroidectomized (0.1)	163.5 ± 7.8 (60)	18.1 ± 0.3 (10)	6.50 ± 0.63 (10)
P	<0.01	<0.01	<0.001
Thyroidectomized (1.8)	201.6 ± 7.8 (60)	19.5 ± 0.3 (10)	10.31 ± 0.41 (12)
P	NS	NS	NS
Thyroidectomized (25)	171.7 ± 4.7 (90)	19.2 ± 0.2 (10)	11.94 ± 0.77 (15)
P	<0.01	NS	NS

[a] Rats were thyroidectomized after weaning and injected daily ip with different doses of L-T_4 for 40 days.
[b] The size of the rats corresponds to the length from the snout to the base of the tail.
[c] Mean ± SEM, with the number of animals in each group shown in parentheses.
[d] Corresponds to the difference between each group and the intact controls.

Glucose removal from blood and glucose space

There were no significant differences in the removal of radioactive glucose from blood (Fig. 1) in thyroidectomized rats given 0, 0.1, or 1.8 μg T_4 compared to that in controls, while glucose removal was significantly enhanced in thyroidectomized rats given 25 μg T_4. Linear regression analysis showed the half-life of labeled blood glucose to be very similar in thyroidectomized rats treated with 0 (9.17 ± 0.57 min), 0.1 (9.21 ± 0.70 min), or 1.8 μg T_4 (7.55 ± 0.66 min) and controls (8.41 ± 0.95 min), while the half-life of labeled blood glucose was significantly decreased in thyroidectomized animals treated with 25 μg T_4 (4.41 ± 0.30 min; $P < 0.0001$ compared to controls). There were no significant differences among the groups in the glucose space. The composite mean glucose space value of all animals from different groups was 47.5 ± 1.9 ml/100 g BW.

Gluconeogenesis in fed animals

The nonglucose radioactivity in blood 5, 15, or 30 min after the injection of [3-^{14}C]pyruvate did not differ significantly among the groups (data not shown). The appearance of [^{14}C]glucose in blood, both in absolute amounts and specific activity (Table 2), were significantly enhanced 30 min after the administration of the tracer in untreated thyroidectomized rats compared to intact controls, while no differences were observed among the other groups.

Gluconeogenesis in response to short periods of starvation (Table 2)

Intact controls. A significant increase in the appearance of labeled glucose in blood and blood glucose-specific radioactivity occurred 5 min after the injection of [3-

^{14}C]pyruvate in intact controls starved for 3 h. The appearance of labeled glucose in blood and glucose-specific radioactivity were also significantly increased 30 min after the injection of [3-^{14}C]pyruvate in control rats starved for 6 h as well as 5, 15, and 30 min after the injection of [3-^{14}C]pyruvate in controls starved for 24 h. Radioactivity in liver glycogen was significantly increased 30 min after the injection of labeled pyruvate in

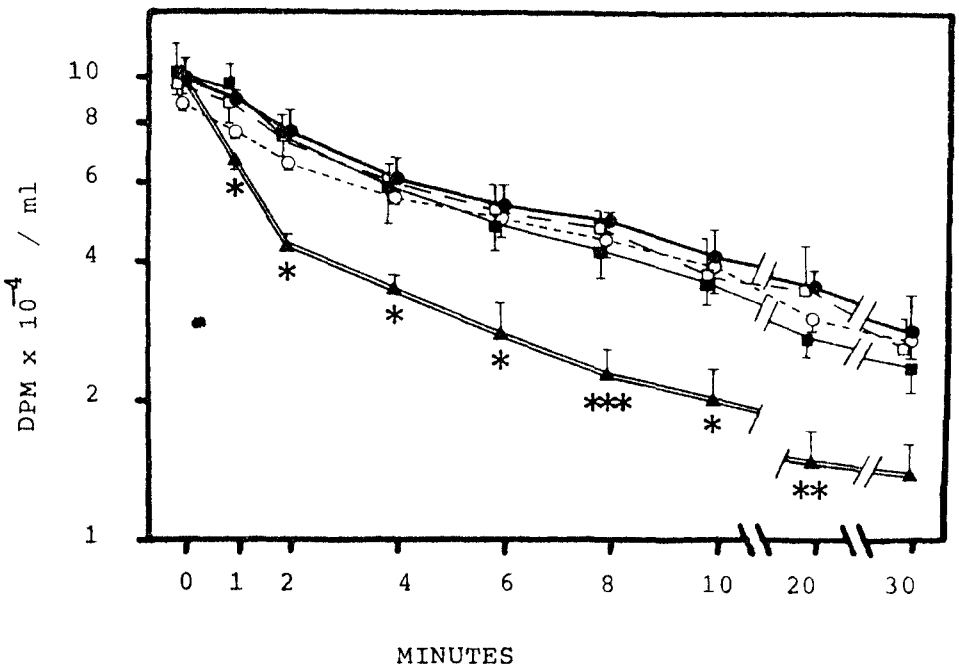

FIG. 1. Effect of thyroidectomy and treatment with L-T_4 on blood [^{14}C]glucose disappearance. Rats were thyroidectomized after weaning and injected ip with 0 (○–○), 0.1 (□— —□), 1.8 (■—■), or 25 (▲=▲) μg L-T_4/100 g BW for 40 days. The animals were compared with intact controls (●—●). The rats were anesthetized with ethyl ether-O_2/CO_2 and injected with 10^7 dpm [*U*-^{14}C]glucose/200 g BW via the femoral vein. Blood was collected at different times from a cannula inserted in the carotid artery. Results correspond to the mean ± SEM of four or five animals per group. The *P* values of the differences between each group and the intact controls are shown by *asterisks* (no asterisk, NS; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

TABLE 2. Effect of thyroidectomy and treatment with L-T_4 on gluconeogenesis in response to food deprivation in rats

Group[a]	Dose of L-T_4 (μg/100 g BW/day)	Hours of starvation	Appearance of radioactive glucose in blood: Formation (dpm × 10^{-4}/100 g BW) 5 min	15 min	30 min	Specific radioactivity (dpm × 10^{-4}/mg) 5 min	15 min	30 min	Appearance of radioactive glycogen in liver: Formation (dpm × 10^{-4}/100 g BW) 30 min	Specific radioactivity (dpm × 10^{-4}/mg) 30 min
Intact controls	0	0	43 ± 10[b]	145 ± 14	212 ± 20	838 ± 178	2,326 ± 537	3,070 ± 325	5,768 ± 1,657	35 ± 11
		3	115 ± 28*	180 ± 31	244 ± 52	2,100 ± 230**	3,241 ± 623	3,824 ± 597	4,573 ± 512	53 ± 10
		6	76 ± 12	185 ± 34	407 ± 56*	2,083 ± 181**	3,754 ± 694	5,999 ± 801**	11,600 ± 3,298	160 ± 43*
		24	146 ± 23**	412 ± 65**	628 ± 126*	3,580 ± 704**	7,411 ± 839***	10,586 ± 1,674**	36,039 ± 5,954**	10,106 ± 1,424***
Thyroidectomized	0	0	166 ± 85	232 ± 64	531 ± 78++	1,701 ± 685	3,114 ± 870	5,291 ± 398++	4,195 ± 417	135 ± 38
		3	212 ± 23	213 ± 49+	342 ± 81	2,043 ± 530	3,274 ± 897	5,528 ± 1,714	3,204 ± 1,141	78 ± 33
		6	116 ± 31	184 ± 65	436 ± 100	2,991 ± 815	3,803 ± 1,108	7,815 ± 1,809	4,681 ± 1,343	460 ± 153
		24	232 ± 56	636 ± 107*	1,365 ± 280*	5,453 ± 965*	13,505 ± 1,646‡**	19,573 ± 2,308‡**	17,922 ± 6,944	7,293 ± 2,548*
	0.1	0	46 ± 14	124 ± 38	270 ± 56	941 ± 284	2,198 ± 630	3,789 ± 701	3,309 ± 1,064	48 ± 26
		3	38 ± 11	129 ± 28	277 ± 18	771 ± 175	2,435 ± 441	4,059 ± 431	2,995 ± 1,374	32 ± 12
		6	81 ± 25	195 ± 43	444 ± 97	2,084 ± 449*	4,141 ± 950	6,762 ± 1,498	4,761 ± 796	243 ± 102
		24	89 ± 14	246 ± 38	726 ± 97**	2,221 ± 254**	6,104 ± 788**	10,796 ± 1,411**	14,129 ± 4,850	4,200 ± 1,363‡*
	1.8	0	25 ± 4	105 ± 12	177 ± 29	818 ± 325	1,723 ± 196	2,815 ± 393	3,797 ± 1,272	22 ± 7
		3	78 ± 14**	200 ± 18**	312 ± 82	1,851 ± 219*	4,282 ± 954*	5,793 ± 777**	5,227 ± 1,307	226 ± 170
		6	76 ± 18*	168 ± 33	330 ± 57*	1,854 ± 432	3,496 ± 649	5,661 ± 950*	6,539 ± 345	83 ± 11
		24	194 ± 22***	493 ± 91**	796 ± 141**	5,401 ± 1,336*	9,928 ± 1,144***	11,730 ± 2,124**	37,004 ± 7,366**	9,956 ± 1,462***
	25	0	58 ± 27	165 ± 68	162 ± 20	945 ± 342	2,756 ± 951	2,668 ± 364	5,860 ± 1,880	255 ± 134
		3	206 ± 56*	392 ± 71++	343 ± 46**	3,836 ± 1,011**	7,383 ± 1,319++	6,092 ± 843**	7,047 ± 1,452	2,115 ± 1,043
		6	115 ± 38	264 ± 83	321 ± 65*	3,146 ± 605*	6,514 ± 1,359	5,661 ± 906*	13,723 ± 3,071	538 ± 108
		24	285 ± 65*	623 ± 174*	642 ± 144*	5,460 ± 951**	10,307 ± 1,680**	9,836 ± 1,221***	5,374 ± 2,803++	4,393 ± 1,974++

Asterisks refer to the *P* values of the differences between starved and fed animals in each group (no asterisk, NS; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). The *P* values of the differences between each group and the intact controls at the same starvation time is shown by: +, $P < 0.05$; ++, $P < 0.01$.

[a] Rats were thyroidectomized after weaning and injected daily with L-T_4 for 40 days. The animals were injected ip with [3-^{14}C]pyruvate (10^7 dpm/0.5 mmol sodium pyruvate/200 g BW), blood was collected, and then the animals were decapitated. Liver sections were frozen in liquid N_2 for glycogen isolation.

[b] Mean ± SEM of five animals per group.

animals starved for 24 h compared to fed animals, while 3 or 6 h of starvation failed to affect the incorporation of radioactivity into liver glycogen.

Untreated thyroidectomized rats. A significant increase in the appearance of labeled glucose in blood occurred 15 and 30 min after the injection of [3-^{14}C]pyruvate into thyroidectomized rats that were not treated with T_4 and were starved for 24 h. However, 3 or 6 h of food deprivation failed to affect the appearance of labeled glucose in the blood. Glucose-specific radioactivity increased significantly after 24 h of starvation but not after 3 or 6 h of fasting in untreated thyroidectomized rats. Starvation failed to affect the incorporation of radioactivity into liver glycogen significantly in the latter animals, while the specific radioactivity of liver glycogen was significantly augmented after 24 h of starvation.

Thyroidectomized rats given 0.1 μg T_4. Fasting for 24 h increased the appearance of [^{14}C]glucose in blood 30 min after the injection of the tracer as well as the specific radioactivity of glucose in blood 5, 15, and 30 min after giving the tracer. Starvation for 6 h significantly augmented the specific radioactivity of glucose in blood 5 min after the injection of the tracer, while 3 h of food deprivation failed to affect the appearance of labeled glucose or the specific radioactivity of glucose in blood. The only effect of fasting on liver glycogen in thyroidectomized rats given 0.1 μg T_4 was a significant increase in specific radioactivity after 24 h of food deprivation.

Thyroidectomized rats given 1.8 μg T_4. As in intact controls, 3 h of starvation increased the appearance of labeled glucose in blood and blood glucose-specific radioactivity. The appearance of labeled glucose in blood and glucose-specific radioactivity were also increased at various times after the injection of labeled pyruvate into intact control rats fasted for 6 h. The appearance of labeled glycogen in the liver and the specific radioactivity of liver glycogen were enhanced 30 min after the injection of labeled pyruvate into thyroidectomized rats given 1.8 μg T_4 and starved for 24 h, while 3 or 6 h of food deprivation failed to affect the incorporation of radioactivity into liver glycogen.

Thyroidectomized rats given 25 μg T_4. As in intact controls, 3 h of food deprivation enhanced the appearance of labeled glucose in blood and blood glucose-specific radioactivity. It is of interest that the increase of labeled glucose in the blood of thyroidectomized rats deprived of food for 24 h and given 25 μg T_4 peaked 15 min after the administration of [3-^{14}C]pyruvate instead of at 30 min, as in control rats. The appearance of labeled glucose in blood and glucose-specific radioactivity were also augmented at various times after the injection of labeled pyruvate into thyroidectomized rats fasted for 6 or 24 h and given 25 μg T_4, as they were in controls. Unlike control rats, however, 24 h of food deprivation failed to affect the incorporation of radioactivity into liver glycogen in thyroidectomized rats given 25 μg T_4, and the increase observed in these animals in glycogen-specific radioactivity in liver after 24 h of starvation was significantly less than that seen in controls.

Discussion

Employing a method widely used (20, 24–26), we measured the conversion of injected [3-^{14}C]pyruvate to radioactive glucose and glycogen as an index of *in vivo* gluconeogenesis and glycogen production in thyroidectomized rats. Any differences in the endogenous pool with which the [^{14}C]pyruvate equilibrated were minimized by calculating the dose of the tracer in terms of the animal's body weight and diluting the labeled substrate with the nonlabeled one. Possible differences in absorption rate of the injected pyruvate into the circulation may not be excluded, although the similar appearance of nonglucose radioactivity in the blood of fed animals indicates that they may be minimal. Our data are discussed in terms of the response to the fasting period in each group rather than by making intergroup comparisons.

Intact control rats showed an enhanced appearance of labeled glucose in the blood as early as 5 min after the injection of the tracer with only 3 h of food deprivation. The enhancement reflects increased *in vivo* gluconeogenesis, since the recycling of newly formed glucose would be minimal 5 min after injection of the tracer (27, 28). Enhancement of gluconeogenesis after this short period without food has also been found in liver slices from normal rats (29) and could be the consequence of altered concentrations of regulatory metabolites (16, 29–31).

The rate of net glucose synthesis was unchanged in the fed untreated thyroidectomized rats. This finding does not contradict the fact that gluconeogenetic enzyme activity is reduced in the liver of thyroidectomized rats (4, 7, 9, 13), since glycolysis is also reduced in thyroidectomized animals (32–36) and there is a balanced equilibrium between these pathways which maintains blood glucose and liver glycogen at normal levels (15, 16, 37).

Signs of metabolic imbalance appeared in food-deprived, untreated, thyroidectomized rats. Neither 3 nor 6 h of fasting enhanced the rate of gluconeogenesis. Furthermore, the rise in labeled glucose in the blood 15 and 30 min after the injection of the tracer in untreated thyroidectomized rats deprived of food for 24 h seemed due to a decreased rate of utilization of newly formed glucose more than to augmented glucose synthesis. The reduced activation of gluconeogenesis in the thyroidectomized rats even after 24 h of food deprivation is supported by the fact that, in these animals, the formation

of [^{14}C]glucose was not augmented 5 min after injection of the tracer, a time when the radioactivity values in blood were mainly dependent on the synthesis of [^{14}C] glucose and were minimally influenced by changes in the rate of its utilization. This explanation agrees with the reduction of glucose formation seen in 24- to 30-h starved thyroidectomized rats (13) and with the rapid fall in liver glycogen content and blood glucose concentration observed in untreated, 24-h starved, thyroidectomized rats (16).

The inability of untreated thyroidectomized fasting rats to enhance gluconeogenesis may not be attributed to a lack of change in regulatory factors such as plasma FFA or liver acetyl-coenzyme A, which actually increase in this preparation (15, 16). Therefore, gluconeogenetic enzymes in these animals must be unable to respond to such stimulating effectors.

The gluconeogenetic response to starvation was also delayed in thyroidectomized rats injected with 0.1 μg T_4, although other metabolic parameters were normalized in these animals (15, 16) which demonstrates their different sensitivities to small changes in thyroid hormone availability.

Daily injections of 1.8 μg T_4 in thyroidectomized rats are known to normalize the circulating level of thyroid hormones (15, 16). In this study, the rates of gluconeogenesis in these animals showed that the absence of the thyroid hormones and not the removal of the thyroid gland was responsible for the metabolic alterations.

The appearance of labeled glucose in the blood after the injection of labeled pyruvate in fed thyroidectomized rats given 25 μg T_4 was similar to that seen in controls, while utilization of newly formed glucose was augmented in these thyroidectomized rats. These results suggest that gluconeogenesis was enhanced in these animals. Our findings are in agreement with those (32, 34, 38–40) reporting augmented turnover of glucose in hyperthyroid animals with a parallel enhancement in the activity of both glycolytic and gluconeogenetic enzymes in rats treated with T_4 (3, 10, 11, 41), and with the enhanced gluconeogenesis in perfused livers from T_4-treated rats (12).

The formation of radioactive glucose from labeled pyruvate was enhanced after only 3 h of food deprivation in thyroidectomized rats given 25 μg T_4, a time at which no significant changes occur in the concentrations of acetyl-coenzyme A, citrate, or glycogen in the liver or FFA in the plasma of these animals (16). Consequently, the enhancement of gluconeogenesis after a short period of starvation occurs independently of changes in these potential effectors in thyroidectomized rats given 25 μg T_4. The fact that the curve for the appearance of radioactive glucose in blood rapidly reached a plateau and that the incorporation of radioactivity in liver glycogen was reduced after 24 h of food deprivation in thyroidectomized rats given 25 μg T_4 suggests that augmented glucose turnover was maintained throughout the fast in the hyperthyroid animals. Thus, although prolonged starvation leads to an enhancement of adipose tissue lipolysis in hyperthyroid animals (42–45), their fat stores are low, so that an enhanced utilization of glucose and a compensatory enhancement of gluconeogenesis supported by the release of amino acids from tissue proteins (46) appear to be necessary for their survival during food deprivation.

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