EXTRATHYROIDAL ACTIONS OF PITUITARY THYROTROPIN: EFFECTS ON THE CARBOHYDRATE, LIPID AND RESPIRATORY METABOLISM OF RAT ADIPOSE TISSUE *

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The thesis that pituitary thyrotropin (thyroid-stimulating hormone, TSH) affects multiple facets of intrathyroidal metabolism and that changes in iodine economy may constitute merely one expression of these alterations (1, 2) is supported by many published reports. These are summarized in Table I. Only data derived by the direct addition of TSH to *in vitro* systems have been tabulated since, herein, the possible metabolic adaptations to changes in thyroid blood flow need not be considered.

The *in vitro* approach has demonstrated that a variety of the oxidative, assimilative and metabolic functions of surviving thyroid tissue are stimulated when TSH is introduced into media containing serum, or other potential organic substrates (3–8) (Table I, A). However, TSH also alters the respiration, hydration, and cytostructural lipids of thyroid tissue even in simple saline media (1, 9–12) (Table I, B). The occurrence of the latter phenomena in the absence of exogenous organic metabolites has prompted the suggestion that TSH *primarily* initiates a realignment of *existing* intrathyroidal pathways and/or a mobilization of *preformed* intrathyroidal substrates (5).

In order to find an extrathyroidal counterpart for this postulated sequence, the effects of TSH upon adipose tissue have been examined. The inquiry was stimulated by the suggestions of Engel, Engel and McPherson that the generalized metabolic action of pituitary hormones may mimic their effects upon target glands (13, 14). In the present studies it has been demonstrated that TSH effects net lipolysis in rat epididymal adipose tissue *in vitro* and that the lipolytic event is accompanied by augmented oxygen consumption and assimilation of glucose. Moreover, specific changes in the disposition of differentially-labeled glucose were effected. Since some of these phenomena could be reproduced by simply supplementing suspending media with albumin-complexed, long chain fatty acids, the experiments suggest that the mobilization of endogenous fatty acids may constitute one of the primary events in the TSH-induced changes in the oxidative and assimilative function of adipose tissue.

MATERIALS AND METHODS

1. Preparation of tissues and media. Epididymal adipose tissue was excised from rats weighing 200 to 280 g (Sprague-Dawley descendants obtained from Charles River Laboratories, Brookline, Mass.). Animals were allowed free access to food (Purina laboratory pellets) and water until the time of sacrifice by stunning, decapitation and exsanguination. Fat pads were exposed individually and divided in half so that a single donor rat was employed to fill four experimental vessels. Duplicate vessels were filled with proximal and distal halves of opposite fat pads in order to randomize anatomical-biological variation in paired control and experimental flasks. In a few studies, individual fat pads were cut into 3 segments so that 6 vessels could be filled with tissues from a single donor animal. Weights of incubated adipose segments ranged from 150 to 300 mg; for any single experiment, weights in individual flasks were adjusted to agree within ± 15 mg.

The following suspending media were employed. *a*) Modified Krebs-Ringer-bicarbonate (KRB): 0.120 M NaCl, 0.005 M KCl, 0.0012 M $MgSO_4$, 0.0008 M $CaCl_2$, 0.0012 M KH_2PO and 0.025 M $NaHCO_3$ in an atmosphere of 95 per cent O_2 — 5 per cent CO_2; or *b*) modified Krebs-Ringer-Tris-phosphate (KRT-P): 0.131 M NaCl, 0.005 M KCl, 0.0012 M $MgSO_4$, 0.0008 M $CaCl_2$ and 0.009 M Tris (hydroxymethyl) aminomethane buffer (pH 7.4) and 0.001 M sodium phosphate buffer (pH

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TABLE I

The effects of added thyroid-stimulating hormone (TSH) upon the intermediate metabolism of thyroid tissue in vitro

A. Effects in supplemented systems (i.e., incubation in serum or in saline media *supplemented* with organic substrates):

1. Increased consumption of oxygen (QO_2) within first hour (3–5).
2. Increased QO_2 and/or preservation of respiratory activity after prolonged incubation (4, 6).
3. Increased assimilation of glucose (5, 7).
4. Increased formation of $C^{14}O_2$ and lipid-C^{14} from glucose-U-C^{14} (5).
5. Formation of $C^{14}O_2$ from glucose-1-C^{14} greater than from glucose-6-C^{14} (7,8).
6. Increased incorporation of inositol-C^{14} into phosphoinositide (5).

B. Effects in nonsupplemented systems (i.e., incubation in simple saline media *without* added organic substrates):

1. Increased QO_2 within first hour (1).
2. Preservation of respiratory activity after prolonged incubation (9).
3. Preservation of water content during prolonged incubation (9, 10).
4. Increased incorporation of orthophosphate-P^{32} into phospholipids (1, 11, 12); sparing the "alkali-stable" phospholipids (12); and selectively affecting cephalins and phosphoinositide (1).

7.4) in an atmosphere of 100 per cent O_2. The KRT-P medium was employed to obtain a bicarbonate-free system containing physiological concentrations of inorganic phosphorus (3 mg per 100 ml) (15) and has been previously demonstrated to support the effects of TSH upon surviving thyroid preparations (1, 5). Enhancement of the glucose metabolism of adipose tissue by bicarbonate *in vitro* has been described by others (16, 17).

In some studies the media were modified to contain: *a*) unlabeled glucose (dextrose, National Bureau of Standards) ± D-glucose-1-C^{14} (lot 307D) or D-glucose-6-C^{14} (lot 341E, Volk Radiochemical Co.) to a final glucose concentration of 2 mg per ml ± 0.2 μc C^{14} per ml; *b*) dialyzed crystalline bovine albumin (Armour, lots V 68802 and V 68204) to a final albumin concentration of 20 mg per ml; or *c*) albumin-complexed sodium oleate (oleic acid, Nutritional Biochemicals Corp.) or sodium palmitate (palmitic acid, Fisher Scientific Co.) to a final concentration of 2 to 8 μmoles free fatty acids (FFA)[1] and 20 mg of dialyzed albumin per ml of suspending medium. In concentrations of 20 mg per ml, the albumin contributed 0.16 to 0.22 μmoles of titratable acid (presumably FFA) to each ml of suspending medium. Supplementation of the albumin with additional quantities of specific fatty acids was effected by a modification of the method of Bragdon and Gordon (20).

For studies of hormonal effects, the following preparations were employed.[2] *a*) ACTH: oxycellulose-adsorbed adrenocorticotropic hormone (ACTH, Wilson Laboratories, lot 104529, 130 USP units per mg; and lot 101900, 103 USP units per mg, from Dr. S. W. Hier). *b*) TSH: commercial "Thytropar" (Armour Laboratories, lots 2402 and U-3810, approximately 1 USP unit per mg; Armour Laboratories lot IRW, 1.5 to 2 USP units per mg, from Dr. S. Steelman); mouse tumor TSH [lot PC 11-113-B1, 7 USP units per mg, from Drs. R. W. Bates and P. G. Condliffe of Bethesda, Md. (21)]; beef TSH [lot PC 9-113 B3, "approximately 30 USP units per mg," from Drs. R. W. Bates and P. G. Condliffe (21)]; and beef thyrotropins, b + c [lot C-5-48-3, 15 to 20 USP units per mg, from Drs. J. G. Pierce and M. E. Carsten of Los Angeles, Calif. (22, 23)]. The beef preparations of Bates and Condliffe and Pierce and Carsten constitute the purest TSH that is currently available. The mouse tumor TSH is derived from transplanted pituitary tumors of athyreotic mice (21), a source in which the presence of other pituitary hormones should be minimal.

Lyophilized hormone preparations were weighed at the time of individual experiments with a Cahn electromagnetic balance, accurate to 1 μg, and dissolved in suspending media for addition to reaction vessels just prior to the start of incubation.

2. Incubation procedures. Incubation for 90 to 180 minutes at 38° C was performed in the Warburg apparatus (shaking rate, 104 cycles per minute) with conventional 15 ml Warburg vessels, or in the Dubnoff metabolic incubator (shaking rate, 90 to 100 cycles per minute) with rubber-stoppered Warburg vessels or 25 ml weighing bottles modified for gassing through needle vents. Two ml of suspending media was employed in the weighing bottles. In the Warburg vessels, 3.0 ml of suspending media was employed. These were placed directly in the main compartment or apportioned between

[1] The term free fatty acid, FFA, will be used in this manuscript as synonymous with nonesterified fatty acid, NEFA (18), and unesterified fatty acid, UFA (19).

[2] The author is greatly indebted to the various investigators whose generosity in sharing their limited supplies of highly purified hormone preparations made these studies possible.

the main compartment (2.7 ml) and side-arms (0.3 ml) for experiments involving timed "tip-in" of hormone. Details of gassing and manometry have been described elsewhere (24).

At the end of incubation, tissues were touched lightly to filter paper to remove adherent medium and were frozen on solid CO_2. Media were removed and processed directly or stored at − 18° C for analysis on the following day. In experiments with glucose-C^{14}, processing of flasks at the end of incubation was modified to permit quantitative evolution of $C^{14}O_2$ into 0.5 M Hydroxide of Hyamine 10X [3] in methanol.

3. Analyses. In most experiments, final values were derived as the average of duplicate vessels. Although such replication minimized the number of possible observations from a single donor animal, it was felt that this was offset by the heightened accuracy thus afforded with an intrinsically variable biological preparation. In addition, all analyses of suspending media were performed with a minimum of two separate aliquots from each vessel.

For measurement of glucose concentration, protein-free filtrates of suspending solutions were prepared by precipitation with $Ba(OH)_2$ − $ZnSO_4$ and processed by the Nelson modification of the Somogyi method (25). Free fatty acids (FFA) in the media were estimated by the method of Dole (18), save for the substitution of Nile Blue A as the indicator solution. In a few instances (see below), the procedure of Gordon (26) was employed. Tissue FFA were measured by directly homogenizing the frozen segments in ground glass homogenizers with Dole extraction mixture and subsequent titration of heptane extracts as described by White and Engel (27).

For assay of $C^{14}O_2$, the alkaline Hyamine was quantitatively transferred into counting vials containing methanol: toluene (12: 88 vol/vol) and 0.4 per cent 2,5-diphenyloxazole (PPO),[4] and 0.005 per cent 1,4-*bis* 2- (5-phenyloxazolyl)-benzene (POPOP).[4] Specimens were counted in an automatic Packard liquid scintillation counter.

4. Expression of results. Oxygen consumption was estimated in terms of microliters per hour per milligram of initial wet weight (QO_2). Net assimilation of glucose, expressed as micrograms per milligram of initial wet weight was calculated on the basis of the difference in the final glucose concentrations in vessels incubated with and without tissues, and a known volume of suspending medium within each vessel. Net changes in the FFA of the suspending media were similarly derived and expressed as micromoles FFA per gram of initial wet weight of tissue. Where tissue FFA were examined directly, values were related to the initial wet weight of the adipose segments. Oxidation of radioactive glucose into $C^{14}O_2$ was quantitated on the basis of recovered C^{14} and the specific activity of the glucose in the initial suspending medium. Results were expressed as micrograms of glucose-C^{14} per milligram of initial wet weight of tissue. In early experiments, attempts to use the weight of the dried "fat-free" residue of the adipose tissue as

[3] Purchased from Packard Instrument Company, Inc., La Grange, Ill.

[4] Purchased from Pilot Chemicals Co., Watertown, Mass.

TABLE II

The effect of thyroid-stimulating hormone (TSH) upon lipolysis in rat adipose tissue in vitro

Expt.* no.	Medium†			Time after "tip-in"‡	Final FFA				FFA $\frac{\text{tissue}}{\text{medium}}$ §	
					Medium		Tissue			
	Type	Alb.	Gluc.		−TSH	+TSH	−TSH	+TSH	−TSH	+TSH
				min	*μmoles/ml*		*μmoles/g*			
1	KRT–P	−	−	20	nd‖	nd	2.26	6.87	nd	nd
				60	nd	nd	2.33	7.22	nd	nd
				120	nd	nd	2.41	15.40	nd	nd
2	KRT–P	−	+	20	nd	nd	2.03	7.11	nd	nd
				120	nd	nd	1.76	7.53	nd	nd
3	KRT–P	+	−	150	0.41	1.04	3.75	10.58	9.1	10.2
4	KRT–P	+	+	150	0.30	0.97	3.05	9.22	10.2	9.5
5	KRT–P	+	+	20	0.25	0.31	2.25	9.24	9.0	29.8
				130	0.22	0.77	1.42	8.46	6.4	11.0
6	KRT–P	+	+	20	0.18	0.30	1.67	10.23	9.3	34.1
				120	0.17	0.81	1.29	11.10	7.6	13.7

* Each experiment number refers to studies with adipose tissue from a single rat. In experiments in which only single control and TSH values are listed (cf. 3 and 4), the data represent the averages of duplicate vessels.

† KRT–P refers to modified Krebs-Ringer-Tris-phosphate not supplemented (−) or supplemented (+) with 20 mg/ml of crystalline bovine albumin (Alb.) and/or 2 mg/ml of glucose (Gluc.). Main compartment of Warburg vessels filled with 2.7 ml of appropriate KRT–P and side-arms filled with 0.3 ml of same medium containing no TSH (−TSH) or 200 ug/ml (i.e., 1.4 USP units/ml) of Bates-Condliffe mouse tumor TSH (+TSH). Gas phase, 100% O_2.

‡ Side-arms "tipped" into main compartments following temperature-equilibration, gassing, and 40 to 60 minutes of control incubation. Final concentration of TSH in "+TSH" vessels was 20 μg/ml (i.e., 0.14 USP units/ml).

§ See text.

‖ None detectable.

TABLE III

The effects of various preparations of TSH upon the release of FFA into the suspending medium during incubation of rat adipose tissue

Medium *			Incubation		TSH†			No. of rats	FFA release	p
Type	Alb.	Gluc.	Volume	Duration	Type					
			ml	*hrs*		*μg/ml*	*U/ml*		*μmoles/g ± SE*	
KRB	+	−	2	1.5	0	0	0	9	2.00 ± 0.32	
			2	1.5	IRW	500	0.75–1.0	2	7.66	‡
			2	1.5	R2402	500	0.50	2	12.64	‡
			2	1.5	BC-beef	16	~0.50	2	6.05	‡
			2	1.5	Tumor	36	0.25	3	5.29§	‡
KRB	+	+	2	1.5	0	0	0	11	−0.02 ± 0.16	
			2	1.5	R2402	1,000	1.0	2	8.58	‡
			2	1.5	R2402	500	0.50	2	10.67	‡
			2	1.5	BC-beef	16	~0.50	2	4.68	‡
			2	1.5	Tumor	36	0.25	3	6.24§	‡
			2	1.5	R2402	100	0.10	2	9.67	‡
			2	1.5	Tumor	10	0.07	2	7.96	‡
KRB	+	+	3	3.0	0	0	0	2	−1.05	
			3	3.0	PC-beef	5	0.07–0.10	1	9.11	
			3	3.0	Tumor	5	0.035	1	7.25	
KRT-P	+	−	3	3.0	0	0	0	2	2.64	
			3	3.0	Tumor	20	0.14	2	12.07	
KRT-P	+	+	3	3.0	0	0	0	10	−0.14 ± 0.26	
			3	3.0	BC-beef	5	~0.17	1	8.80	‡
			3	3.0	Tumor	20	0.14	5	10.54 ± 0.78	<0.01
			3	3.0	PC-beef	5	0.07–0.10	1	7.59	‡
			3	3.0	Tumor	10	0.07	2	9.65	‡
			3	3.0	Tumor	5	0.035	1	7.58	‡

* As in Table II.

† IRW and R2402 refer to commercial preparation Thytropar (Armour) assaying 1.5 to 2.0 and 1.0 USP units/mg, respectively; 0 = no TSH. BC-beef = Bates-Condliffe purified beef TSH (PC 9-113B3) assaying "about 30 USP units/mg." PC-beef = Pierce-Carsten purified beef thyrotropins b + c (C-5-48-3) assaying "15–20 USP units/mg." Tumor = Bates-Condliffe mouse tumor TSH (PC 11-113-B1) assaying "7 USP units/mg." Lyophilized TSH preparations were dissolved in suspending media to obtain the concentrations in μg/ml and USP units/ml (U/ml) listed above and on subsequent tables.

‡ Differs from mean by more than two SD.

§ Analyses for FFA performed by the method of Gordon (26).

the reference standard for analytical results (i.e., by extracting each segment with 2:1 chloroform-methanol after incubation, or a representative segment prior to incubation) did not significantly enhance reproducibility or reduce animal-to-animal variation.

Statistical methods for assessing significance of differences between paired control and experimental vessels have been described in other publications from this laboratory (5, 28).

RESULTS

1. The effect of TSH upon the lipid metabolism of adipose tissue. As shown in Table II, the "tipping-in" of mouse tumor TSH was followed by a profound augmentation in the FFA pool of the incubated adipose tissue. Near-plateau values were effected within 20 minutes. The increased concentration of intracellular FFA occurred in albumin-free as well as in albumin-containing systems. However, whereas no FFA appeared in the medium in the absence of albumin, the presence of extracellular albumin was accompanied by a progressive release of FFA into the suspending medium. Since the absolute tissue content of FFA did not appreciably change during the 20 minute to 3 hour interval, the rising level of FFA within the medium presumably reflected continuing lipolysis in excess of the rate of FFA utilization by adipose tissue. Thus, during serial observations, tissue to medium concentration gradients for FFA, i.e., FFA [tissue (μmoles/g)/medium (μmoles/ml)], progressively declined and approached values observed in the control vessels (Table II).

The magnitude of concentration differentials for FFA between tissue and medium seemed to be

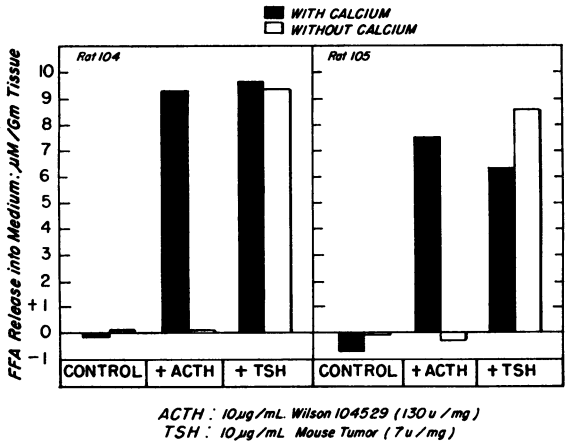

FIG. 1. THE INDUCTION OF LIPOLYSIS IN RAT ADIPOSE TISSUE BY ACTH AND TSH *IN VITRO*: THE RELATIONSHIP OF EXTRACELLULAR IONIC CALCIUM TO HORMONE ACTION. Concentrations of ACTH or TSH in the final reaction mixture are denoted by μg/ml. The potencies of the hormone preparations employed in these experiments are expressed in terms of USP units per mg (U/mg).

largely conditioned by the concentration of albumin in the suspending fluid [i.e., the abundance of extracellular acceptor sites for FFA (29)]. Accordingly, in subsequent documentation of the effects of various TSH preparations upon FFA, media containing 20 mg per ml of crystalline bovine albumin were employed, and lipolytic potencies were compared solely on the basis of change in the FFA concentration of the medium. Results of experiments with five different preparations of TSH in concentrations of 0.01 to 1.0 USP unit TSH per ml are summarized in Table III. In KRB as well as KRT-P, all preparations of TSH effected a release of FFA into the suspending medium which exceeded by at least threefold the modest FFA production occasionally observed in paired control vessels.

Despite the positive findings with highly purified TSH preparations, the possibility still remained that the stimulation of lipolysis was induced by trace contamination with other pituitary principles. Of the adenohypophyseal factors that are known to activate lipolysis, only ACTH has sufficient *in vitro* activity on a weight basis to qualify as such a contaminant. ACTH was excluded by the technique of Lopez, White and Engel (30) (Figure 1). Six segments of epididymal adipose tissue from single donor rats were incubated individually for 1 hour in Ca^{++}-free KRB containing 1 mg per ml glucose, 20 mg per ml albumin, and 1 mg per ml EDTA.[5] Thereafter, tissues were lightly blotted and introduced into similar Ca^{++}-free or Ca^{++}-containing KRB solutions which had been further supplemented with nothing (control), or 10 μg per ml Oxycel ACTH (ACTH), or 10 μg per ml mouse tumor TSH (TSH). Two of four such experiments are pictured in Figure 1. In confirmation of Lopez and associates, the activity of ACTH was not manifest in the absence of extracellular ionic calcium. To the contrary, the activity of TSH was unaffected.

2. The effects of TSH upon the respiratory and carbohydrate metabolism of adipose tissue. The introduction of TSH (Figure 2) effected a prompt augmentation of QO_2 that was manifest at the earliest manometric readings (i.e., 10 to 20 minutes) following "tip-in." Concomitantly, the assimilation of glucose from the suspending medium was increased. Results of experiments performed with five different preparations of TSH in concentrations exceeding 0.030 USP unit per ml are shown in Table IV. Respiratory enhancement was invariable. It occurred in albumin-free as well as in albumin-containing KRT-P and did not require glucose for initiation. However, in both systems, the presence of glucose not only enhanced QO_2 in control flasks, but also caused a greater and more sustained stimulation of oxygen consumption by TSH. Effects upon glucose assimilation were more variable, presumably, in part, because of the analytical limitations of deriving values on the basis of minor changes in the suspending medium. Nonetheless, for every type of medium, the effects of TSH in concentrations of 0.03 to 1.0 USP unit per ml (Table IV and the 10 μg per ml values from Table V) were highly significant (p, 0.01 or less). To assess whether some parallelism existed between the effects of TSH upon glucose and respiratory metabolism, a rank order correlation coefficient (31) was derived for the 25 experiments with KRT-P in which both parameters were measured concurrently. The correlation between the absolute increase in QO_2 (ΔQO_2) and the absolute change in the assimilation of glucose (Δ glucose assimila-

[5] Ethylenediamine tetraacetic acid dihydrate.

tion) proved to be of marginal statistical significance (R, 0.470; p, $< 0.05 > 0.01$).

For glucose and QO_2, as for the release of FFA, no clear dose-response relationship could be delineated when individual animals which had been examined at a single TSH concentration in the 0.03 to 1.0 USP unit per ml range were intercompared (Tables III and IV). Therefore, experiments were performed with smaller quantities of TSH. Results of five experiments are shown in Table V. As little as 1 μg per ml (0.007 USP unit per ml) of mouse tumor TSH elicited detectable augmentation of lipolysis, glucose assimilation, and oxygen consumption. Moreover, when paired adipose segments from a single donor animal were compared, the effects of 1 μg per ml upon all parameters were smaller in magnitude than the changes that were induced by 10 μg per ml. In the latter experiments, the fourth piece of adipose tissue was incubated concurrently with

TABLE IV

The effect of various preparations of TSH upon oxygen consumption and glucose assimilation by rat adipose tissue in vitro

Expt.* no.	Medium†			TSH‡			QO_2			Glucose assimilation		
	Type	Alb.	Gluc.	Type			−TSH	+TSH		−TSH	+TSH	
					μg/ml	U/ml	μl/mg/hr		Δ	μg/mg		Δ
7*	KRB	+	+	R2402	1,000	1.0				1.29	1.69	0.40
8*		+	+	R2402	1,000	1.0				2.24	1.94	−0.30
9*		+	+	BC-beef	16	0.5				0.15	1.12	0.97
10*		+	+	BC-beef	16	0.5				0.21	0.98	0.77
11*		+	+	Tumor	36	0.25				0.13	0.42	0.29
12*		+	+	Tumor	36	0.25				0.60	0.59	−0.01
13*		+	+	Tumor	36	0.25				0.34	0.81	0.47
14*		+	+	PC-beef	5	0.07–0.10				1.73	3.04	1.31
15*		+	+	Tumor	10	0.07				0.58	1.38	0.80
16*		+	+	Tumor	10	0.07				0.64	0.65	0.01
17*		+	+	Tumor	5	0.035				2.47	3.25	0.78
18	KRT-P	−	−	U 3810	330	0.33	0.105	0.139	0.034			
		−	+				0.118	0.224	0.106	0.36	1.39	1.03
19		−	−	U 3810	330	0.33	0.112	0.188	0.076			
		−	+				0.130	0.243	0.113	0.25	1.16	0.91
20		−	+	Tumor	50	0.35	0.166	0.271	0.105	0.92	1.57	0.65
21		−	−	Tumor	20	0.13	0.126	0.154	0.028			
		−	+				0.131	0.203	0.072	0.34	0.56	0.22
22		−	−	Tumor	20	0.14	0.141	0.164	0.023			
		−	+				0.169	0.210	0.041	0.76	0.41	−0.35
23		−	+	Tumor	10	0.07	0.112	0.174	0.062	0.51	1.31	0.80
24		−	+	Tumor	10	0.07	0.077	0.148	0.071	0.70	1.14	0.44
25		−	+	Tumor	10	0.07	0.147	0.172	0.025	0.52	0.96	0.44
26		−	+	Tumor	10	0.07	0.171	0.239	0.068	0.66	0.79	0.13
27		−	+	Tumor	10	0.07	0.090	0.140	0.050	0.30	0.99	0.69
28		−	+	Tumor	10	0.07	0.117	0.138	0.021	1.41	0.66	−0.75
29		−	+	Tumor	10	0.07	0.127	0.220	0.093	1.14	1.39	0.25
30	KRT-P	+	+	BC-beef	5	0.15	0.127	0.178	0.051	0.49	1.27	0.78
31		+	+	Tumor	20	0.14	0.200	0.265	0.065	0.92	0.76	−0.16
32		+	−	Tumor	20	0.14	0.210	0.278	0.068			
		+	+				0.361	0.519	0.158	1.69	3.50	1.81
33		+	−	Tumor	20	0.14	0.183	0.192	0.009			
		+	+				0.214	0.263	0.049	1.09	0.82	−0.27
34*		+	+	Tumor	20	0.14	0.263	0.319	0.056			
35*		+	+	Tumor	20	0.14	0.155	0.253	0.098			
36*		+	+	PC-beef	5	0.07–0.10	0.123	0.183	0.060	0.52	1.42	0.90
37		+	+	Tumor	10	0.07	0.139	0.151	0.012	0.69	1.20	0.51
38		+	+	Tumor	10	0.07	0.200	0.242	0.042	1.52	1.78	0.26
39		+	+	Tumor	10	0.07	0.097	0.140	0.043	0.46	1.32	0.86
40*		+	+	Tumor	5	0.035	0.123	0.170	0.047	0.49	0.78	0.29

* Experiments in which media were also assayed for FFA.

† KRB refers to modified Krebs-Ringer-bicarbonate and KRT-P to modified Krebs-Ringer-Tris phosphate not supplemented (−) or supplemented (+) with albumin (Alb.) and/or glucose (Gluc.), as in Table II. In all KRB studies except 14 and 17, volume of suspending medium was 2.0 ml and incubation was for 90 minutes at 38° C. In experiments 14 and 17, and all KRT-P studies, suspending medium consisted of 3.0 ml and incubation was for 3 hours. Gas phase, KRB, 95% O_2–5% CO_2; KRT-P, 100% O_2.

‡ TSH, as in Table III. U 3810 represents commercial preparation Thytropar (Armour) assaying 1 USP unit/mg; μg/ml and U/ml (USP units/ml) refer to final concentration in reaction mixture.

TABLE V

The parallelism between the effects of TSH upon lipid, respiratory and carbohydrate metabolism of rat adipose tissue in vitro

Expt. no.	Medium* Type	Alb.	Gluc.	Mouse tumor TSH	Release FFA into medium	QO_2	Glucose assimilation
				μg/ml	μmoles/g	μL/mg/hr	μg/mg
41	KRT-P	+	+	0	−0.14	0.195	1.00
				1.0	3.08	0.231	1.72
42	KRT-P	+	+	0	−0.32	0.127	0.56
				10.0	10.30 [−0.76]†	0.219 [0.127]	1.64 [0.85]
				1.0	3.48	0.176	1.24
43	KRT-P	+	+	0	−1.16	0.281	2.14
				10.0	9.01 [0.26]	0.368 [0.254]	2.46 [1.82]
				1.0	3.55	0.296	2.27
44	KRT-P	−	+	0	nd	0.139	0.88
				10.0	nd	0.199 [0.173]	1.71 [0.77]
				1.0	nd	0.185	1.35
45	KRT-P	−	+	0	nd	0.126	0.65
				10.0	nd	0.212 [0.151]	1.62 [1.22]
				1.0	nd	0.156	1.15

* As in Table II. The μg/ml of TSH refers to the final concentration of Bates-Condliffe mouse tumor TSH (7 U/mg) in the 3.0 ml reaction mixture. Incubation, 3 hours.

† Values in brackets denote results obtained with adipose tissue from the same animals when 10 μg/ml of TSH (Bates-Condliffe mouse tumor) which had been heated at 96° to 98° C for 3 minutes at pH 2 was employed; nd = none detectable.

10 μg per ml of mouse tumor TSH which had been heated (96° to 98° C) for 3 minutes at pH 2. The results of such mild acid-heating upon the lipolytic, respiratory and assimilative potencies of TSH are shown by the values in brackets in Table V. In every instance, the activities of TSH upon lipolysis, oxygen consumption, and glucose assimilation were reduced at least 90 per cent. When comparable experiments were performed with ACTH (Figure 3), no measurable inactivation was produced by mild acid-heating, thus further indicating that the effects of TSH upon lipid, respiratory and carbohydrate metabolism did not result from ACTH contamination.[6]

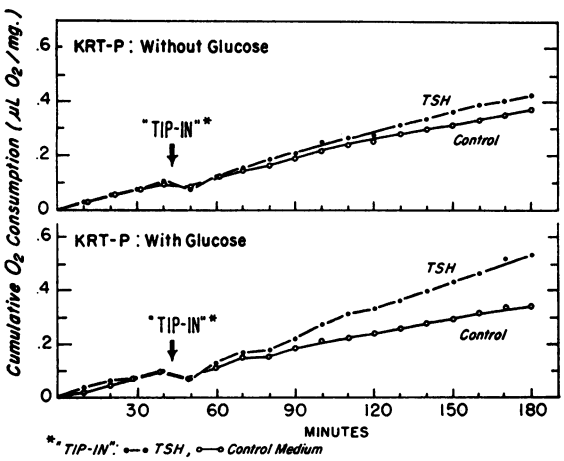

FIG. 2. THE EFFECTS OF TSH UPON THE OXYGEN CONSUMPTION OF RAT ADIPOSE TISSUE: INFLUENCE OF EXTRACELLULAR GLUCOSE. Adipose segments from a single donor animal were employed to fill four vessels. Paired vessels were incubated in KRT-P with or without 2 mg glucose per ml. After control observations, mouse tumor TSH in sufficient amounts to yield 20 μg per ml was tipped into one of each pair of vessels. Comparable quantities of KRT-P without added hormone were tipped into the control flasks.

The actions of TSH upon the differential oxidation of C-1- and C-6-labeled glucose were assessed in five experiments with the three most purified preparations of hormone (Table VI). In KRT-P as well as KRB media, TSH effected minimal changes in the oxidation of glucose-1-C^{14}. However, a two- to sixfold increase in the evolution of $C^{14}O_2$ from glucose-6-C^{14} was produced. As a consequence of the altered disposition of assimilated glucose, the average C-1/C-6 ratio for evolved $C^{14}O_2$ declined from 3.9 in control vessels to 1.2 in the presence of TSH.

3. The effects of free fatty acids upon the respiratory and carbohydrate metabolism of adipose tissue. To evaluate the possibility that some of the effects of TSH upon respiratory and carbohydrate metabolism might result from the increased intracellular availability of FFA, paired segments of adipose tissue were incubated *a*) in

[6] The use of mild acid-heating as a means of differentiating between TSH and ACTH by selective inactivation was suggested by Dr. Robert W. Bates.

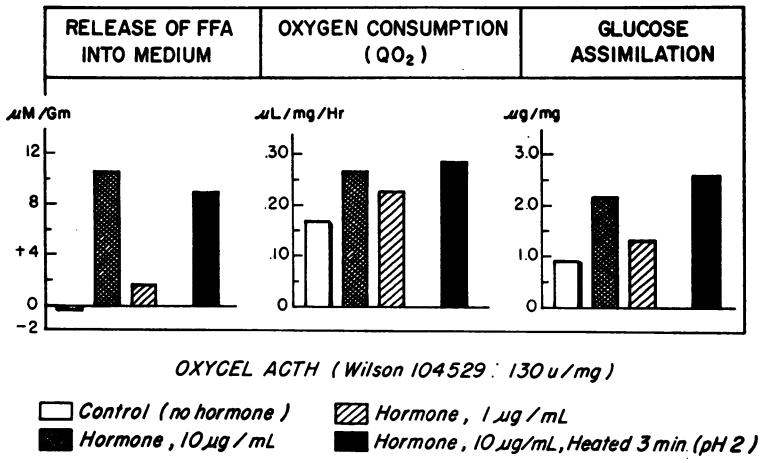

FIG. 3. DOSE-RESPONSE RELATIONSHIPS IN THE ACTIONS OF ACTH UPON ADIPOSE TISSUE: EFFECTS OF MILD ACID-HEATING UPON ACTH POTENCY. Adipose segments from a single donor animal were employed to fill four vessels containing respectively: a) KRT-P plus glucose, 2 mg/ml, plus albumin, 20 mg/ml (control); *b*) as in *a* but supplemented with 10 μg/ml ACTH; *c*) as in *a* but supplemented with 1 μg/ml ACTH; *d*) as in *a* but supplemented with 10 μg/ml of ACTH which had been heated (96°to 98° C) for 3 minutes at pH 2.

control systems with media containing glucose, albumin and 0.18 to 0.22 μmole per ml of titratable fatty acids, and *b*) in experimental systems in which the FFA of these media had been supplemented with oleate or palmitate. As judged by final tissue/medium concentration differentials for FFA (Table VII), the addition of FFA to the suspending medium resulted in a true increase in the intracellular pool of FFA (i.e., tissue/medium ratios exceeded unity). Moreover, in confirmation of the findings of others (32–34), cellular utilization of the FFA was demonstrated by failure to recover all of the added palmitate or oleate. In association with this disposition of FFA, the oxygen consumption of surviving adipose segments was uniformly enhanced (Figure 4). Mean stimulation of QO_2 above control values averaged 33 ± 6 per cent[7] in 13 experiments. Effects of the added oleate or palmitate upon the differential oxidation of C-1- and C-6-labeled glucose were investigated in nine experiments (Table VIII). The oxidation of glucose C-1 was increased slightly in eight of the nine studies, whereas the formation of $C^{14}O_2$ from glucose-6-C^{14} was augmented in every instance, and, proportionally, to a greater extent. Results in KRB and KRT-P systems were qualitatively similar.

[7] Mean ± standard error of the mean.

TABLE VI

The effect of various preparations of TSH upon the oxidation of glucose-1-C^{14} and glucose-6-C^{14} by rat adipose tissue

Expt. no.*	Medium*	TSH*	Oxidation of glucose carbon to CO_2					
			C-1		C-6		C-1/C-6	
			−TSH	+TSH	−TSH	+TSH	−TSH	+TSH
			μg C^{14}-glucose/g initial wet weight					
14	KRB	PC-beef	556	575	143	856	3.9	0.7
17	KRB	Tumor	967	919	207	543	4.7	1.7
30	KRT-P	BC-beef	141	142	36	207	3.9	0.7
36	KRT-P	PC-beef	152	268	42	181	3.6	1.5
40	KRT-P	Tumor	96	139	26	91	3.7	1.5

* Numbers refer to the same experiments as in Table IV. All media contained glucose (2 mg/ml) and crystalline bovine albumin (20 mg/ml) and either were (+TSH) or were not (−TSH) supplemented with 5μg/ml of TSH as above.

TABLE VII

The disposition of albumin-bound free fatty acids (FFA) during incubation of rat adipose tissue in glucose-containing media

Expt. no.	Medium FFA* Type	Initial	Final	Δ	Tissue FFA Final		Net† Δ	FFA $\frac{\text{tissue}}{\text{medium}}$
				μmoles	*μmoles*	*μmoles/g*	*μmoles*	
46	Oleate	1.78	1.35	− 1.29	0.40	2.43	−0.89	1.8
47	Oleate	8.10	3.84	−12.78	6.54	30.37	−6.24	7.9
48	Oleate	8.28	3.94	−13.02	6.03	29.48	−6.99	7.5
49	Oleate	8.35	3.94	−13.23	9.70	46.13	−3.53	11.7
53	Palmitate	1.62	1.22	− 1.20	0.30	1.50	−0.90	1.2

* Medium consisted of 3 ml KRT–P containing glucose (2 mg/ml), crystalline bovine albumin (20 mg/ml), and FFA complexed to the albumin in concentration designated as "initial" above. Concentration of titratable fatty acids in the albumin prior to addition of palmitate or oleate was 0.18 to 0.22 μmoles/ml.

† Net Δ (μmoles): minimal estimate of the net quantity of FFA which disappeared from the system during 3 hours of incubation at 38° C and in an atmosphere of 100% O_2. The value was derived as the difference between the change in the FFA of the suspending medium (i.e., medium Δ; [Initial-Final] × 3), and the tissue FFA (μmoles) at the end of incubation. Corrections for alterations in the endogenous FFA of the tissue during incubation were not made.

Thus, C-1/C-6 ratios for $C^{14}O_2$ evolution were invariably reduced by the enrichment of suspending media with naturally occurring free fatty acids.

DISCUSSION

The present studies indicate that thyrotropin must be added to the growing list of pituitary principles (17, 27, 35–43) which can exert a direct influence upon the metabolism of surviving rat adipose tissue. At least superficially, it appears that the *in vitro* response to several of these hormones is quite similar. Thus, with TSH, as with ACTH (27, 39, 43) and growth hormone (STH) (27), lipolysis is activated *in vitro*. Like ACTH (37, 42, 43) and STH (17, 36), TSH also promotes a selective increase in the oxidation of the sixth carbon of glucose. Finally, TSH enhances the net assimilation of glucose, and even in the absence of glucose, stimulates the oxygen consumption of adipose tissue. During the preparation of this manuscript, comparable properties have been reported for ACTH and for a purified bovine growth hormone preparation (41, 43). Whether the seeming uniformity of response reflects contamination of the available ACTH, TSH and STH preparations with one another or even with other pituitary components cannot be answered with complete finality. For growth hormone, the contaminant possibility is rendered tenable by the large quantities that are required for manifest action *in vitro* (17, 27, 36, 41). However, in the case of ACTH and TSH, the hormone requirements are of much smaller magnitude. Moreover, fairly effective dissociation of ACTH and TSH can be achieved. In the present studies it has been demonstrated that a degree of mild acid-heating, which does not affect ACTH, diminishes the activity of TSH upon all of these aspects of adipose tissue metabolism and that the lipolytic potency of TSH, unlike that of ACTH (30), is not contingent upon the avail-

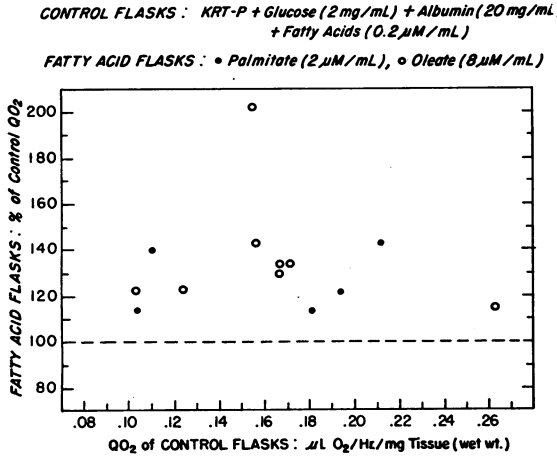

FIG. 4. EFFECTS OF NATURALLY OCCURRING FATTY ACIDS UPON THE OXYGEN CONSUMPTION OF RAT ADIPOSE TISSUE. "Fatty acid flasks" denote vessels in which control media have been further supplemented with palmitate (2 μmoles/ml) or oleate (8 μmoles/ml). Values for oxygen consumption by adipose tissue in the enriched systems have been expressed as a percentage of the QO_2 which was observed in the paired control flasks. By this convention, 100 per cent (i.e., the dotted line) denotes no difference between control and experimental flasks.

ability of extracellular ionic calcium. In addition, corroborative experiments were performed with the highly purified beef thyrotropins of Bates and Condliffe, and Pierce and Carsten. Their isolation by sequential cationic and anionic exchange chromatography should exclude more basic components, such as ACTH, and minimize the inclusion of more acidic products (21–23). Furthermore, in the TSH derived from mouse pituitary tumors, one might anticipate negligible contamination with other pituitary hormones even in the initial starting material (21). Thus, the likelihood that the above effects of TSH can be ascribed to any known pituitary principles besides TSH would seem to be vanishingly small. Nonetheless, until the precise structure of all adenohypophyseal hormones has been elucidated, the possibility cannot be excluded that the seemingly similar intracellular activities of ACTH, TSH and perhaps STH in adipose tissue are mediated by a common peptide component [in the manner of the pigment effects of α-MSH and β-ACTH (44)] and that apparent differentiations by selective inactivations affect only those configurational characteristics of the intact hormone that govern transcellular transport.

The multiple effects of TSH upon the carbohydrate, lipid and respiratory metabolism of the epididymal fat pad prompted some effort to distinguish between primary and secondary events. The possibility that the increased intracellular availability of FFA might be implicated was subjected to preliminary examination. Instead of augmenting FFA from within by inducing lipolysis with TSH, the absolute quantities of both esterified and unesterified intracellular fatty acids were increased from without by adding oleate or palmitate to suspending media containing albumin and glucose. This resulted in a reduplication of the increased oxygen consumption and the selective alteration in the oxidation of differentially labeled glucose.[8] Concurrently, Cahill, Leboeuf and Flinn reported similar effects of added palmitic acids upon the oxidation of glucose (49) and Lynn has shown that added butyrate stimulates "glucose uptake, respiration and fat synthesis from glucose" in adipose tissue (50). The precise mechanism by which changes in carbohydrate and respiratory economy are effected by fatty acids remains obscure and whether *every* aspect of TSH action in adipose tissue can be reproduced in this fashion must await more detailed chemical dissection. However, if the latter

[8] Parenthetically, it should be noted, that in some of these experiments, concentrations of added fatty acids were employed that might obtain during periods of lipid mobilization *in vivo* (45). The ensuing alterations in the disposition of glucose may have intriguing implications for the pathogenesis of some of the complications which are observed in such clinical states as diabetes mellitus, wherein chronic, intermittent elevations of plasma FFA occur (46). In the least, however, the findings that FFA can influence the carbohydrate metabolism of isolated adipose tissue warrant consideration in the use of the epididymal fat pad for bioassay of insulin-like activity (47), and may, conceivably, be involved in some of the anomalous results with plasma from totally pancreatectomized animals (48).

TABLE VIII

The effect of high concentrations of albumin-bound FFA upon the oxidation of glucose-1-C^{14} and glucose-6-C^{14} by rat adipose tissue

Expt. no.	Medium*	FFA* Type		Oxidation of glucose carbon to CO_2: C-1 −FFA	C-1 +FFA	C-6 −FFA	C-6 +FFA	C-1/C-6 −FFA	C-1/C-6 +FFA
			μmoles/ml	*μg C^{14}-glucose/g initial wet weight*					
48	KRT–P	Oleate	8.28	209	433	87	234	2.4	1.8
49	KRT–P	Oleate	8.35	105	121	70	127	1.5	0.9
50	KRT–P	Oleate	8.04	46	58	17	33	2.7	1.8
51	KRT–P	Oleate	8.12	149	151	78	153	1.9	1.0
52	KRB	Oleate	6.06	471	633	146	354	3.2	1.8
53	KRT–P	Palmitate	1.62	136	167	53	86	2.6	1.9
54	KRT–P	Palmitate	2.20	250	350	38	130	6.6	2.7
55	KRB	Palmitate	1.74	362	264	110	132	3.3	2.0
56	KRB	Palmitate	1.74	253	274	110	174	2.3	1.6

* Numbers refer to the same experiments as in Table VII. All media contained glucose (2 mg/ml) and crystalline bovine albumin (20 mg/ml). The albumin either was (+FFA) or was not (−FFA) complexed with added fatty acids as above. Concentration of titratable fatty acids in the −FFA media was 0.18 to 0.22 μmoles/ml.

obtains, as seems quite likely, then the potentiation of TSH action upon adipose tissue QO_2 which was observed in the presence of glucose suggests that a supporting role must be assigned to other processes directly or indirectly concerned with the disposition of glucose.

Although many other isolated tissues have been examined (1, 7), the fat pad seems to be the only preparation in which an extrathyroidal action of TSH can be demonstrated. From the available data, it is hard to assess whether the effects of TSH upon adipose tissue *in vitro* are of physiological significance *in vivo*. The lower limits of responsiveness of adipose tissue were not explored but changes were routinely elicited with concentrations of TSH that are only five to ten times greater than the upper levels of TSH in normal human plasma (21). In the intact animal, the possibility that TSH conjointly activates the thyroid and mobilizes oxidizable extrathyroidal metabolites in response to increased peripheral metabolic demands has undeniable teleological attraction. Similarly, it would be tempting to ascribe the exophthalmogenic potential of certain thyrotropins to extrathyroidal actions upon the metabolism of retro-ocular lipids [or other components (51)]. However, the mouse tumor TSH which was employed for some of the present experiments has no exophthalmogenic activity (52), and many of the previous observations of generalized metabolic alterations following the administration of TSH (14, 53) were obtained in animals with intact thyroid glands. In view of the prompt rise in plasma FFA after the exhibition of thyroid hormone (54), it appears that athyreotic animals should be employed for assessment of the adipokinetic potential of TSH *in vivo*. Such animals should also receive adequate replacement therapy on the conceivable basis that TSH, like epinephrine (55), may require permissive quantities of thyroid hormone for maximal activation of lipolysis *in vivo*. In this regard, the observations of Hetzel, Charnock and Good may be germane (56). Two of their five human subjects with treated myxedema responded to injected TSH with acute elevations of oxygen consumption although they dismissed the findings as "due to non-specific stress." Re-examination of the entire problem of extrathyroidal TSH actions seems warranted. Of special interest might be investigation of adipose tissue metabolism in mice with thyrotropin-secreting tumors of the pituitary, since plasma TSH levels are chronically elevated in these animals (21).

Motivating all of the present efforts was the hope that clues might evolve relevant to the author's sustaining interest in the mechanism of action of TSH within the thyroid. On the basis of previous studies, we had postulated that the primary intrathyroidal action of TSH is independent of organic extracellular substrates but that concomitant or secondary changes in thyroid assimilative function are necessary for sustained TSH action (5). Insofar as TSH within the fat pad: *a*) stimulates oxygen uptake in simple saline media; *b*) promotes glucose assimilation in glucose-containing media; and *c*) enhances QO_2 maximally in the presence of extracellular glucose, an extrathyroidal counterpart for the proposed sequence of TSH action within the thyroid has been obtained. Moreover, insofar as TSH activates lipolysis in adipose tissue, a model system has been described wherein intracellular substrate can be augmented by TSH independent of extracellular supply.[9] Indeed, the fact that some of the effects of TSH upon oxygen consumption and glucose oxidation could be reproduced by simple supplementation of suspending media with FFA raises the intriguing possibility that the mobilization of preformed substrate by TSH may, at least in adipose tissue, constitute the progenitor for the changes in respiratory and carbohydrate metabolism. Obviously, caution must be exercised in applying the findings from the fat pad to the problems of TSH action in the thyroid. For any tissue, the consequences of hormone action and substrate mobilization will be conditioned by the subcellular localization of these processes, the nature and abundance of preformed substrate, and the intrinsic biochemical potentialities of that tissue. Thus, in view of the organizational dissimilarities of thyroid and adipose tissue, it is not surprising that certain processes, such as the oxidation of assimilated glucose, are quite differently affected by TSH in these structures. However, within the framework of suggesting new avenues for thyroid investigation,

[9] Although glycogen metabolism has not been studied, analogy to findings with other hormones (35, 38) suggests that a stimulation of glycogenolysis by TSH in adipose tissue also might be anticipated.

the present studies have fulfilled experimental objectives. Whether the data justify more specific extrapolation with regard to the intrathyroidal action of TSH must be deferred for further inquiry.

SUMMARY

1. The actions of pituitary thyrotropin (TSH) upon the metabolism of rat epididymal adipose tissue have been examined. Commercial as well as the most highly purified available preparations of TSH were employed.

2. Addition of all types of TSH to the incubation mixture resulted in an increase in the tissue stores of free fatty acids, and a release of fatty acids into the suspending medium in the presence of extracellular albumin. In contradistinction to the lipolytic effects of equivalent quantities of corticotropin (ACTH), the action of TSH was not contingent upon the presence of extracellular ionic calcium.

3. Concomitant with the induction of lipolysis, TSH effected an enhancement of the oxygen consumption of adipose tissue which was maximal in the presence of extracellular glucose. Assimilation of glucose from all types of suspending media was also promoted by TSH. Heating of TSH for 3 minutes at pH 2 caused profound reduction in its effects upon lipolysis, respiration and glucose assimilation. Such mild acid-heating did not diminish the similar activities of ACTH in adipose tissue.

4. Differentially-labeled glucose-C^{14} was employed to assess the effects of TSH upon the disposition of assimilated glucose. TSH caused a disparate augmentation in the oxidation of glucose carbon-6.

5. Simple supplementation of albumin and glucose-containing media with larger quantities of palmitate or oleate effected comparable alterations in oxygen consumption and glucose oxidation. The parallelisms prompted the suggestion that lipolysis may constitute the progenitor for some of the TSH-induced changes in the metabolism of surviving adipose tissue.

6. Adipose tissue has been analyzed as a model system wherein stimulation of respiratory activity and assimilative capacity may occur coincident with mobilization of preformed endogenous substrate. The possible implications with regard to the intrathyroidal action of TSH have been discussed.

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