

## Relationship between Free Fatty Acid Turnover and Total Body Oxygen Consumption in the Euthyroid and Hyperthyroid States \*

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Many of the signs and symptoms characteristic of the hyperthyroid state can be elicited in closely similar form by the administration of epinephrine or norepinephrine. Both in clinical and in experimental hyperthyroidism, some receptor systems are known to be more sensitive to catecholamines than they are in the euthyroid state (1). It has been frequently suggested, therefore, that the manifestations of hyperthyroidism are to some extent indirect effects, secondary to enhancement of effective sympathetic activity, rather than direct effects of the thyroid hormone itself. There is convincing evidence that at least some of the cardiovascular manifestations of hyperthyroidism are indeed due to enhanced sympathetic activity (2, 3). However, the evidence with regard to the hypermetabolism of the hyperthyroid state is more difficult to evaluate. Some studies have shown a partial correction toward normal by chemical or surgical interference with the activity of the sympathetic system (2, 4-6), whereas other similar studies have shown no such effect (7, 8).

We have reported previously that the calorogenic effect of intravenously infused norepinephrine can be prevented by prior administration of a  $\beta$ -adrenergic blocking agent, pronethalol (9). These studies also showed that norepinephrine-induced fat mobilization was blocked, suggesting a relationship between free fatty acid mobilization and calorogenesis. Because chronic toxicity experiments in rats subsequently showed that pronethalol might have carcinogenic potential, use of this effective blocking agent in man was abandoned in continuing studies. However, Carlson and Orö (10) have shown that intravenously administered nicotinic acid is also capable of blocking catecholamine-induced FFA mobilization. In

the present paper, we report further studies on the relationship between fat mobilization and total body oxygen consumption, as observed in the euthyroid state and in the induced hyperthyroid state and as modified by nicotinic acid. In addition, the effects of nicotinic acid administration on the fat mobilizing and calorogenic action of norepinephrine are described in euthyroid and hyperthyroid subjects.

### Methods

The subjects studied were young, adult, normal volunteers on regular ward diets. Metabolic studies were carried out after a 16-hour fast. The general procedures for placement of intravenous catheters, infusion of palmitate-1- $C^{14}$ , calculation of FFA turnover, oxygen consumption, fractionation of lipids, and measurement of radioactivity were identical to those described in a previous publication (9).

The protocol for studies of the effects of nicotinic acid was as follows: After establishment of a 30-minute baseline control value for oxygen consumption in the metabolic chamber, a 15- to 30-minute intravenous infusion of norepinephrine (Levophed)<sup>1</sup> or epinephrine<sup>2</sup> was given at a rate of 0.15 to 0.3  $\mu$ g of base per kg body weight per minute. The response in oxygen consumption was evaluated by integration of the continuous record during the 15-minute period of the infusion and the 15 minutes following cessation of the infusion. This time period was selected because FFA turnover and oxygen consumption generally return to control values within 30 minutes after the end of such an infusion of catecholamines. When nicotinic acid was used, its administration was started 15 to 30 minutes after the end of the catecholamine infusion, and it was given in five or six divided doses totaling 900 to 1,000 mg. Each dose was given slowly over a period of 2 to 3 minutes.

The effect of nicotinic acid itself on oxygen consumption, in the studies in which it was given following an infusion of catecholamine, was evaluated over the 15-minute period surrounding the last dose of nicotinic acid.

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Thus, these values for oxygen consumption were obtained at least 45 to 60 minutes after the end of the first infusion of catecholamine. The second infusion of catecholamine was started within 10 minutes after the final dose of nicotinic acid. The response to the second infusion of catecholamine was again evaluated by integrating oxygen consumption for the 15 minutes during and the 15 minutes following the catecholamine infusion. When nicotinic acid was administered without prior catecholamine infusion, the response in oxygen consumption was evaluated during the 30 minutes following the final dose. The tabulated measurements of respiratory quotient (RQ) were calculated using values of  $\dot{V}CO_2$  integrated over the same time intervals described above for  $\dot{V}O_2$ .

Control values for FFA level and FFA turnover represent averages of those obtained during the 10 minutes immediately preceding administration of catecholamine or of nicotinic acid. The responses to catecholamines were evaluated from the averages of the values obtained at the time the infusion was stopped and those obtained during the next 10 minutes. The responses to nicotinic acid were evaluated from the average of values obtained at the time of the last nicotinic acid injection and those obtained during the following 15 minutes.

During the initial intravenous dose of 100 mg of nicotinic acid, the subjects all showed pronounced flushing beginning in the face and progressing over the entire body. They reported pronounced sensations of heat in the face and in the extremities, and a few noted transient abdominal discomfort without nausea or vomiting. Two patients noted a tight feeling in the chest and difficulty in drawing a deep breath during this first dose. The four additional doses of 200 mg each were then given at intervals of 5 to 10 minutes. The subjective reactions to these succeeding doses became progressively less severe, and generally the fourth and fifth doses evoked little or no subjective response. The erythema of the skin persisted for at least 20 to 30 minutes, but then began to fade despite the additional large doses of the drug. In most subjects, there was little or no change in pulse rate or blood pressure during nicotinic acid administration.

The response to norepinephrine in the control period was much like that reported previously (9). Systolic

blood pressure rose by an average of 40 mm Hg and diastolic pressure by an average of 31 mm Hg. There was a decided slowing of the pulse, presumably a reflex response to elevated blood pressure. These responses were not significantly altered by the prior administration of nicotinic acid.

Triiodothyronine was generally given orally on the following dosage schedule: 200  $\mu$ g the first day, 300  $\mu$ g per day for 3 days, and then 500  $\mu$ g per day for 5 days. Metabolic studies were carried out on the eighth or ninth day of triiodothyronine administration.

## Results

*Effects of intravenous nicotinic acid in normal subjects.* As shown in Table I, intravenous administration of nicotinic acid rapidly reduced plasma FFA concentrations to very low levels, agreeing with the previous work of Carlson and Orö (10). The control levels, after an overnight fast, averaged 0.50  $\mu$ Eq per ml in the four studies shown and fell to a mean steady-state level of about 0.2  $\mu$ Eq per ml after administration of nicotinic acid. The FFA levels began to fall immediately after the initial dose of 100 mg and had almost reached their new lower plateau level by the time the third dose had been given, that is, within 30 minutes after starting the series of divided doses. The time course of the response in a representative study can be seen in Figure 1. The FFA concentration showed no tendency to return toward normal for at least 30 to 60 minutes after completion of the course of intravenous doses of nicotinic acid. In two studies, later samples were taken, and it was found that somewhere between 1 and 2 hours after the last dose of nicotinic acid, FFA levels had risen again to pretreatment values.

The decrease in FFA level due to nicotinic acid

TABLE I  
*Effects of nicotinic acid on FFA level, FFA turnover, and oxygen consumption in euthyroid subjects*

Patient	FFA concentration		FFA turnover		$\dot{V}O_2$		RQ*	
	Control	After nicotinic acid	Control	After nicotinic acid	Control	After nicotinic acid	Control	After nicotinic acid
	$\mu$ Eq/ml		$\mu$ Eq/min		ml/min			
MRF I	0.47	<0.2			229	232	0.69	0.70
MRF II	0.59	0.23	526	267	225	245	0.77	0.74
EES I	0.34	<0.2			230	246	0.74	0.72
EES II	0.61	0.29	650	280	247	248		

\* RQ = respiratory quotient.

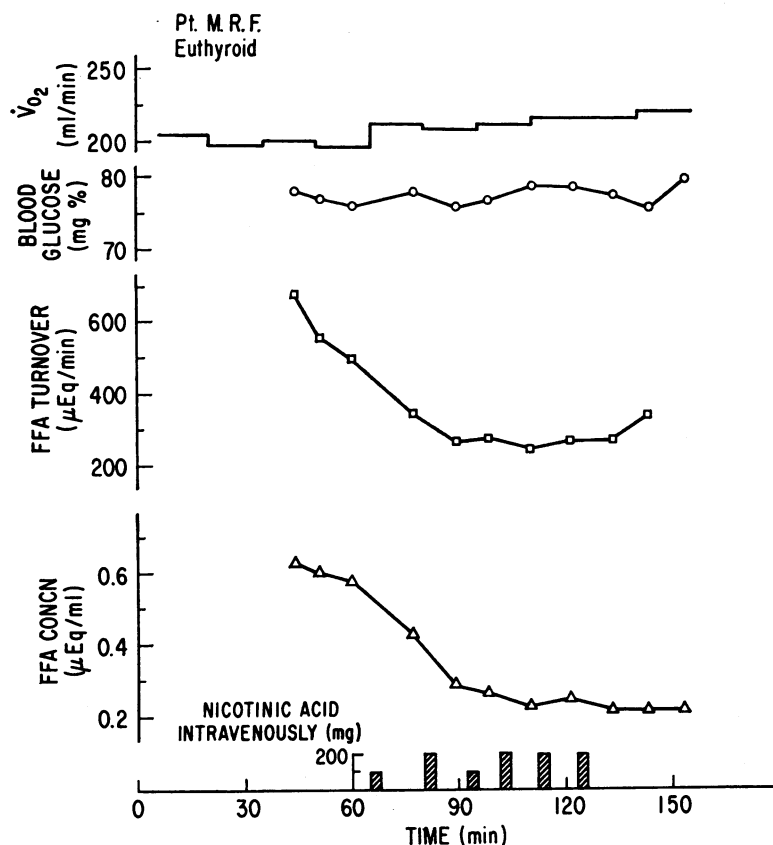


FIG. 1. EFFECTS OF IV NICOTINIC ACID ON OXYGEN CONSUMPTION (TOP), BLOOD GLUCOSE LEVEL (—○—), FFA TURNOVER (—□—), AND FFA CONCENTRATION (—△—) IN A NORMAL, YOUNG, EUTHYROID SUBJECT.

was accompanied by a comparable decrease in FFA turnover in the two studies shown in Table I. As shown in Figure 1 (patient MRF II), the time course of the drop in turnover rate closely paralleled that of the drop in FFA concentration. The administration of nicotinic acid reduced FFA

turnover to about 50% of the control value. Blood glucose levels, determined in two studies, showed no significant changes due to nicotinic acid (see Figure 1).

Oxygen consumption and RQ were not significantly affected by administration of nicotinic

TABLE II  
Effects of nicotinic acid on FFA level, FFA turnover, and oxygen consumption in triiodothyronine-treated subjects

Patient	FFA concentration		FFA turnover		$\dot{V}O_2$		RQ	
	Control	After nicotinic acid	Control	After nicotinic acid	Control	After nicotinic acid	Control	After nicotinic acid
	$\mu\text{Eq/ml}$		$\mu\text{Eq/min}$		$\text{ml/min}$			
MRF	0.80	0.24	827	335	325	327	0.69	0.75
EES	0.75	0.54	1,123	615	322	351	0.69	0.66
EES*	0.80	0.26	2,235	900	328	341	0.73	0.65
JAA	0.90	0.22	670	203	300	304	0.66	0.66
GG	1.35	0.38	3,515	1,030	245	273	0.70	0.65

\* On oral course of nicotinic acid at time of acute study.

TABLE III

FFA concentration and turnover compared in the euthyroid and in the hyperthyroid states in the same subjects

Subject	FFA concentration		FFA turnover		$\dot{V}O_2$	
	$T_3$ -		$T_3$ -		$T_3$ -	
	Control	treated*	Control	treated	Control	treated
	$\mu\text{Eq/ml}$		$\mu\text{Eq/min}$		$\text{ml/min}$	
MRF	0.59	0.80	526	827	200	325
EES	0.61	0.80	650	2,235	247	328

\*  $T_3$  = triiodothyronine.

acid in either euthyroid subjects (Table I) or triiodothyronine-treated subjects (Table II), despite the marked fall in FFA turnover. The quantitative aspects of this dissociation are discussed later in more detail.

*Effects of triiodothyronine ( $T_3$ ) on FFA levels and turnover.* In studies previously reported from this laboratory (9), control values for FFA level and turnover were obtained in young normal subjects under conditions of study exactly like those used here. Combining present data with these earlier data shows, for 13 studies in eight normal subjects, a mean basal FFA concentration of  $0.60 \mu\text{Eq per ml}$  ( $\text{SE} \pm 0.05$ ) and FFA turnover (in 11 studies) of  $361 \mu\text{Eq per minute}$  ( $\text{SE} \pm 42$ ). By comparison, our five studies in four subjects treated with triiodothyronine (Table II) show a mean FFA level of

$0.92 \mu\text{Eq per ml}$  ( $\text{SE} \pm 0.11$ ) and turnover of  $1,674 \mu\text{Eq per minute}$  ( $\text{SE} \pm 530$ ).

Two subjects in the present series were studied when euthyroid and again when hyperthyroid, thus serving as their own controls. As shown in Table III, both FFA level and turnover were higher during treatment with  $T_3$ .

*Effect of intravenous nicotinic acid in subjects treated with triiodothyronine.* As shown in Table II, the effects of nicotinic acid in hyperthyroid subjects were qualitatively like those seen in the euthyroid subjects, i.e., FFA levels and turnover fell markedly, whereas oxygen consumption and RQ showed no significant change. However, the absolute changes in FFA concentrations and turnover, starting as they did from much higher values under the influence of  $T_3$ , were considerably greater than in the euthyroid subjects. Complete data from the studies on subjects MRF and EES are plotted as a function of time in Figures 2 and 3.

*Relationship between FFA turnover and oxygen consumption.* If we assume that these fasted subjects were burning fat exclusively, we can calculate from the measured  $\dot{V}O_2$  the total number of microequivalents of fatty acid being oxidized per minute. Since the RQ were below 0.8 in all cases but one (mean 0.71), carbohydrate must have contributed very little to the metabolic mixture. The contribution of protein, metabolized

TABLE IV

Comparison of calculated rate of fatty acid oxidation\* with observed FFA flux through plasma compartment

Subject	Control period		After nicotinic acid	
	Total fatty acid oxidation calculated from $\dot{V}O_2$ *	Observed total FFA flux through plasma	Total fatty acid oxidation calculated from $\dot{V}O_2$ *	Observed total FFA flux through plasma
	$\mu\text{Eq/min}$	$\mu\text{Eq/min}$	$\mu\text{Eq/min}$	$\mu\text{Eq/min}$
Euthyroid				
MRF	388	526	422†	267
EES	447	650	482†	280
Hyperthyroid				
MRF	631	827	635†	335
EES	625	1,123	682†	615
EES	636	2,235	663	900
JAA	583	670	590†	203
GG	476	3,515	530	1,030

\* Calculation made assuming that only fatty acids are being oxidized (see text for discussion).

† Indicates studies in which calculated total fatty acid oxidation exceeded the observed total flux of FFA through the plasma compartment.

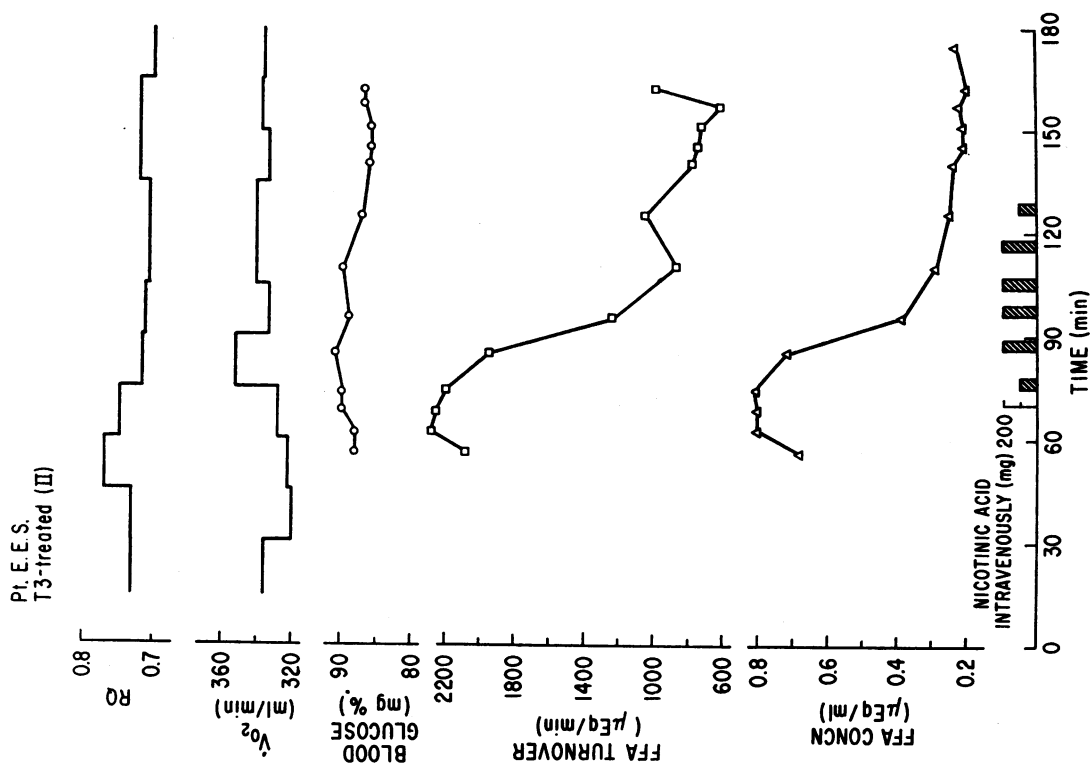


FIG. 3. EFFECTS OF IV NICOTINIC ACID ON  $RQ$ , OXYGEN CONSUMPTION, BLOOD GLUCOSE, FFA TURNOVER, AND FFA CONCENTRATION IN A TRIIODOTHYRONINE-TREATED SUBJECT.

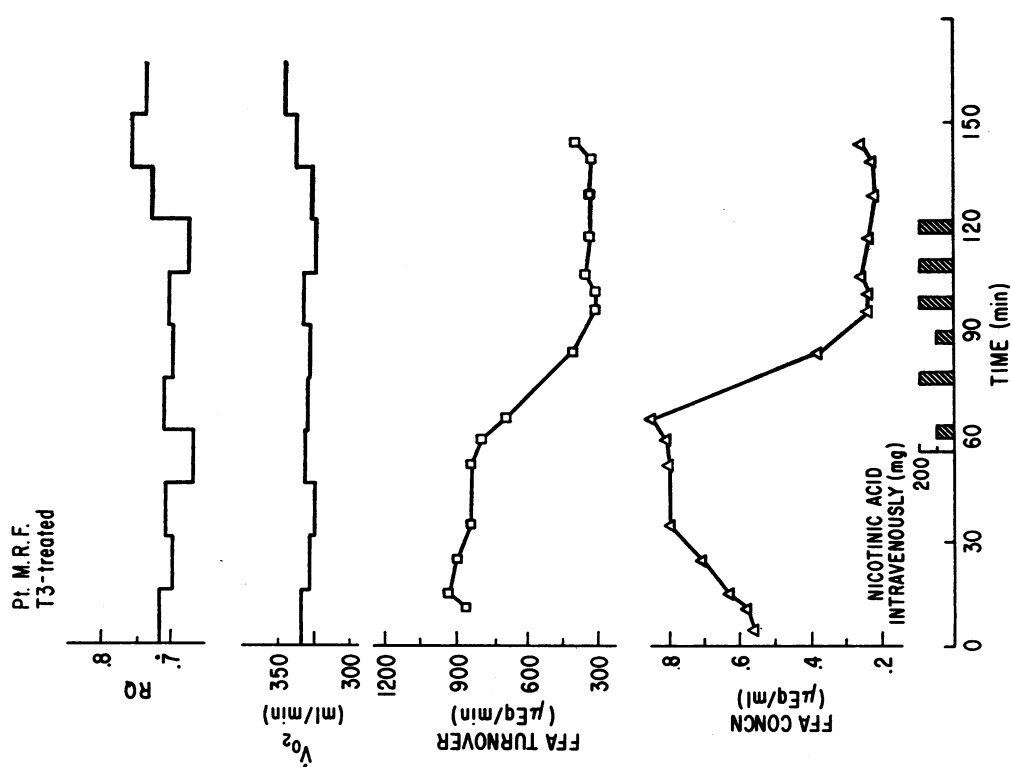


FIG. 2. EFFECTS OF IV NICOTINIC ACID ON RESPIRATORY QUOTIENT ( $RQ$ ), OXYGEN CONSUMPTION, FFA TURNOVER, AND FFA CONCENTRATION IN A TRIIODOTHYRONINE ( $T_3$ )-TREATED SUBJECT.

TABLE V  
Modification of the effects of catecholamines by prior administration of nicotinic acid

Subject	FFA concentration				FFA turnover				Vo <sub>2</sub>			
	Before nicotinic acid		After nicotinic acid		Before nicotinic acid		After nicotinic acid		Before nicotinic acid		After nicotinic acid	
	Control	Catecholamine	Control	Catecholamine	Control	Catecholamine	Control	Catecholamine	Control	Catecholamine	Control	Catecholamine
	$\mu\text{Eq/ml}$	$\mu\text{Eq/ml}$	$\mu\text{Eq/ml}$	$\mu\text{Eq/ml}$	$\mu\text{Eq/min}$	$\mu\text{Eq/min}$	$\mu\text{Eq/min}$	$\mu\text{Eq/min}$	$\text{ml/min}$	$\text{ml/min}$	$\text{ml/min}$	$\text{ml/min}$
Euthyroid												
EES Norepinephrine	0.34	0.76	0.10	0.10	650	900	280	520	230	239 (+4%)	246	245 (0)
EES Norepinephrine	0.61	1.24	0.29	0.30					247	272 (+10%)	248	258 (+4%)
JG Norepinephrine	0.46	1.20	0.14	0.26					208	236 (+13%)	199	212 (+7%)
JG Norepinephrine	0.58	1.08	0.16	0.22					196	244 (+24%)	199	215 (+8%)
DCI Norepinephrine*	0.90	2.08	0.07	0.09					196	221 (+13%)	209	216 (+3%)
Hyperthyroid												
EES Norepinephrine	0.75	1.52	0.54	0.50	1,123	1,995	615	670	322	338 (+5%)	351	354 (+1%)
JAA Epinephrine	0.90	2.00	0.22	0.26	670	1,430	203	290	300	344 (+15%)	304	325 (+7%)

\* Subject received a 30-minute rather than the standard 15-minute infusion of norepinephrine. The dosage rate was 0.2  $\mu\text{g}$  per kg per minute for the first 15 minutes and then 0.3  $\mu\text{g}$  per kg per minute for the last 15 minutes.

with an RQ of about 0.8, cannot be directly assessed. However, from Benedict's studies in fasted subjects (11), we can estimate that oxidation of protein contributed only about 15% of the calories consumed. In Table IV are shown the values calculated in this way for total fatty acid oxidized and, for comparison, the observed values for total FFA flux through the plasma. Before the administration of nicotinic acid, both in the euthyroid and in the hyperthyroid states, the total FFA flux through the plasma was more than enough to account for all of the fat being oxidized and could therefore theoretically have been providing all of the metabolic fuel. The striking finding here is that, in five of seven cases, the turnover of FFA following administration of nicotinic acid fell to a point at which it could not account for the observed total oxygen consumption even if all of the plasma FFA being turned over were being oxidized. Indeed in some cases, oxidation of plasma FFA could have accounted for only about one-half of the total oxygen consumption, and in one case (JAA) only about one-third of it.

*Response to intravenous catecholamines and its modification by pretreatment with nicotinic acid.* The norepinephrine-induced rises in FFA level, FFA turnover, and  $\text{Vo}_2$  during the control period were generally similar to the responses previously reported (9). However, the responses to norepinephrine following the administration of nicotinic acid were significantly modified. Results of seven studies are summarized in Table V. A complete continuous record of values for oxygen consumption and expired carbon dioxide are seen in Figure 4 with the integrated  $\text{Vo}_2$  plotted over 15-minute intervals.

Administration of nicotinic acid, as in the studies described above, caused FFA level and turnover to fall well below control levels, but caused no significant change in  $\text{Vo}_2$  or RQ from the initial pretreatment values.

The second catecholamine infusion, given after the course of intravenous doses of nicotinic acid, raised FFA levels very little or not at all (mean change + 0.03  $\mu\text{Eq}$  per ml). Although there was some increase in FFA turnover in response to the second catecholamine infusion, the increases were smaller, and peak values reached were still

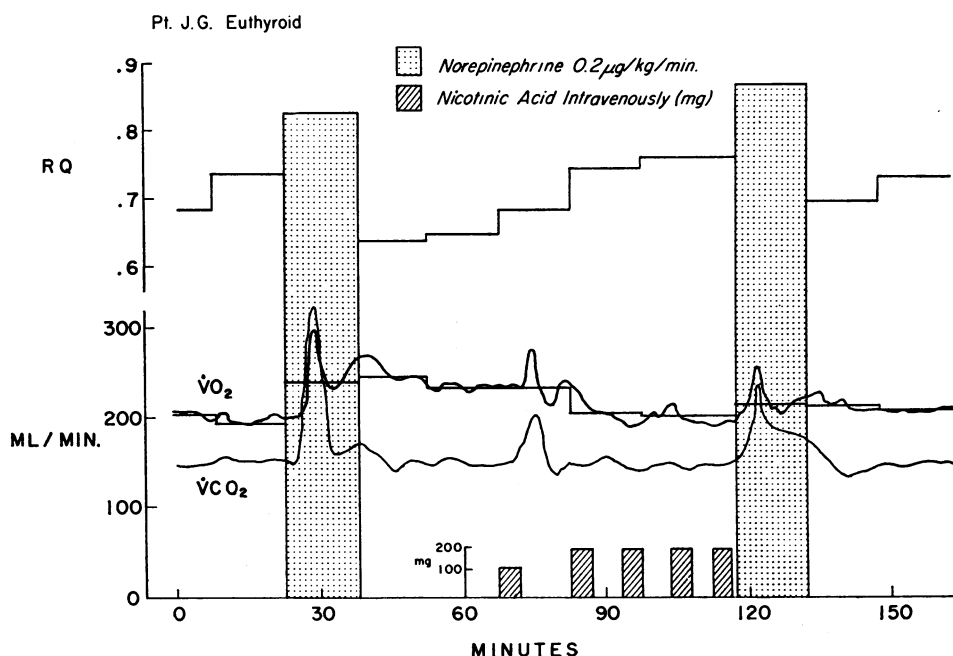


FIG. 4. EXAMPLE OF CONTINUOUS RECORD OF  $\dot{V}O_2$  AND  $\dot{V}CO_2$  OBTAINED IN METABOLIC CHAMBER DURING STUDIES LIKE THOSE SUMMARIZED IN TABLE V. The  $\dot{V}O_2$  obtained by integration over 15-minute periods is superimposed upon the continuously recorded  $\dot{V}O_2$ .

lower than the basal values obtained before administration of drugs.

In every case, the increase in  $\dot{V}O_2$  in response to the second infusion of catecholamine was smaller than that seen with the first infusion. In some subjects, the rise in  $\dot{V}O_2$  in response to the first infusion was quite small, and in these cases it is difficult to be certain of the significance of the modifying effect of the nicotinic acid. In the four studies in which the initial response was greater than 10% (mean + 16%), however, the lower response under the influence of nicotinic acid (mean + 6%) is quite clear. The integrated  $\dot{V}O_2$  records in three cases are shown in Figures 5, 6, and 7. These records bring out the greater duration of the  $\dot{V}O_2$  response to catecholamine before nicotinic acid administration compared to the postnicotinic acid response. As explained under Methods, the tabulated data include only the first 15-minute period after the end of the catecholamine infusion.

As shown in Figure 7, the effect of epinephrine on FFA mobilization was blocked as effectively as that of norepinephrine. On the other hand, the hyperglycemic response did not appear to be altered.

The changes observed in respiratory quotient in response to catecholamine infusion were not significantly affected by prior administration of nicotinic acid. A rise in RQ generally occurred during the infusion, followed by a fall to below control values during the 15 minutes immediately following the cessation of the infusion. These changes are consistent with some degree of hyperventilation during the infusion followed by compensatory retention of  $CO_2$ .

*Effects of glucose on hypermetabolic effect of  $T_3$ .* Glucose very effectively reduces plasma FFA levels by suppressing release from adipose tissue depots (12). We wished to determine whether large doses of glucose would reduce FFA levels in the hyperthyroid state and, if so, whether this would influence metabolic rate. First, the effects of glucose were studied in the euthyroid state and then, in the same two subjects at the same doses, in the hyperthyroid state.

Blood glucose levels rose to values over 200 mg per 100 ml, and FFA levels fell below 0.2  $\mu$ Eq per ml. The respiratory quotient rose steadily, but there was no fall in  $\dot{V}O_2$ . Instead,  $\dot{V}O_2$  tended to rise toward the end of the glucose infusion, although this may be attributable in part

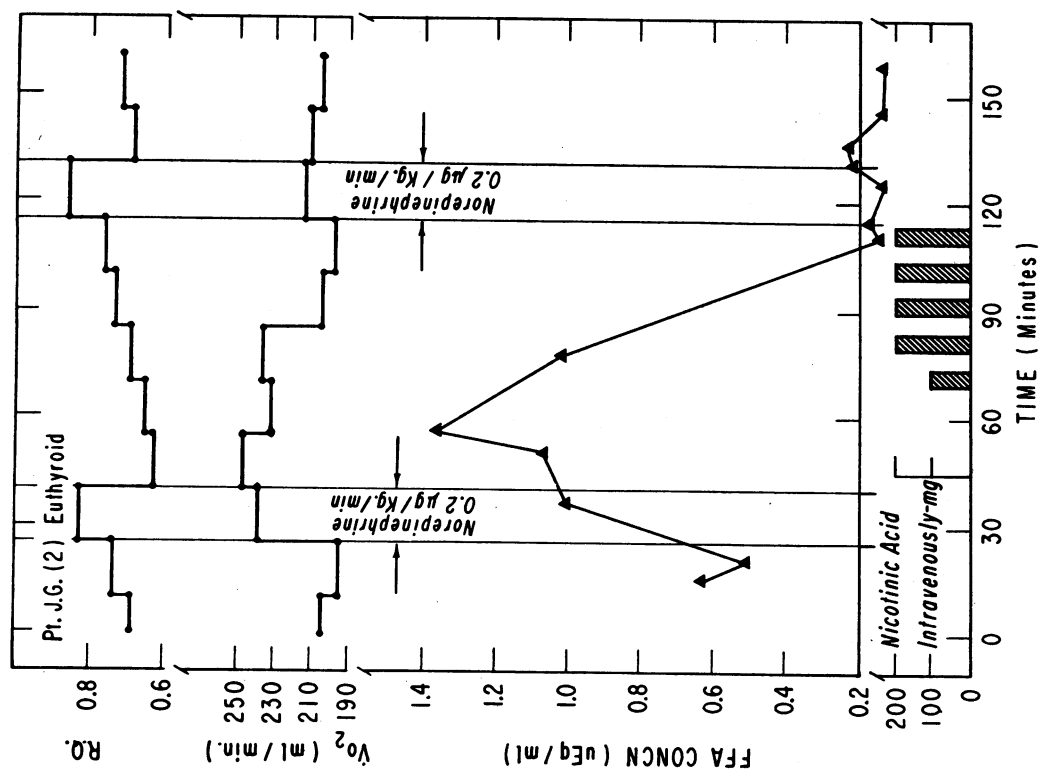


FIG. 5. MODIFICATION BY NICOTINIC ACID OF THE FFA-MOBILIZING AND CALORGENIC EFFECTS OF NOREPINEPHRINE IN A NORMAL EUTHYROID SUBJECT (JG).

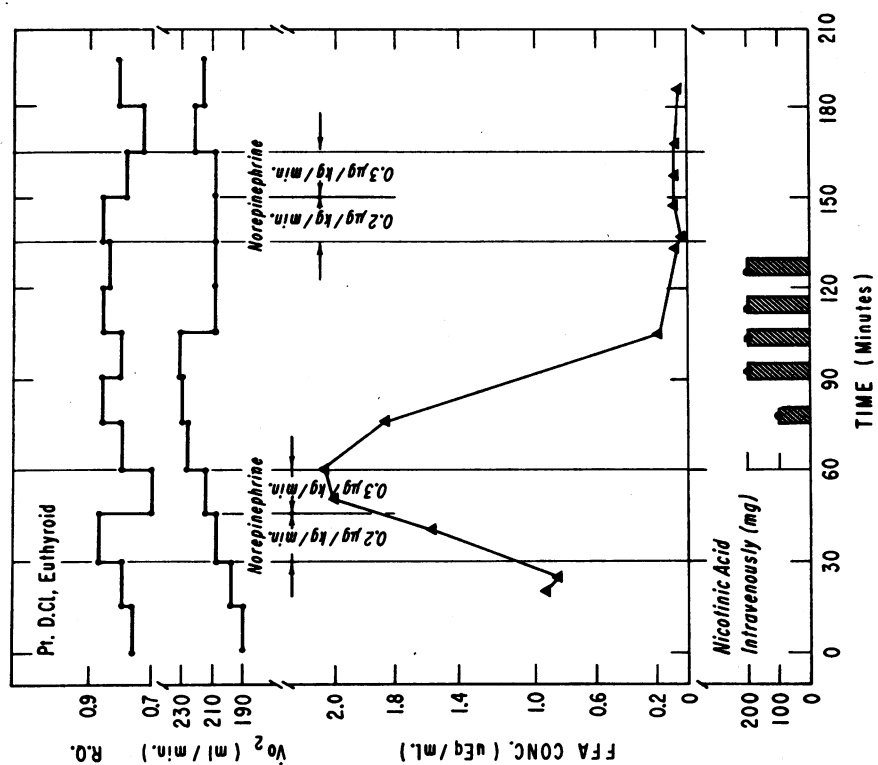


FIG. 6. MODIFICATION BY NICOTINIC ACID OF THE FFA-MOBILIZING AND CALORGENIC EFFECTS OF NOREPINEPHRINE IN A NORMAL EUTHYROID SUBJECT (DCI).



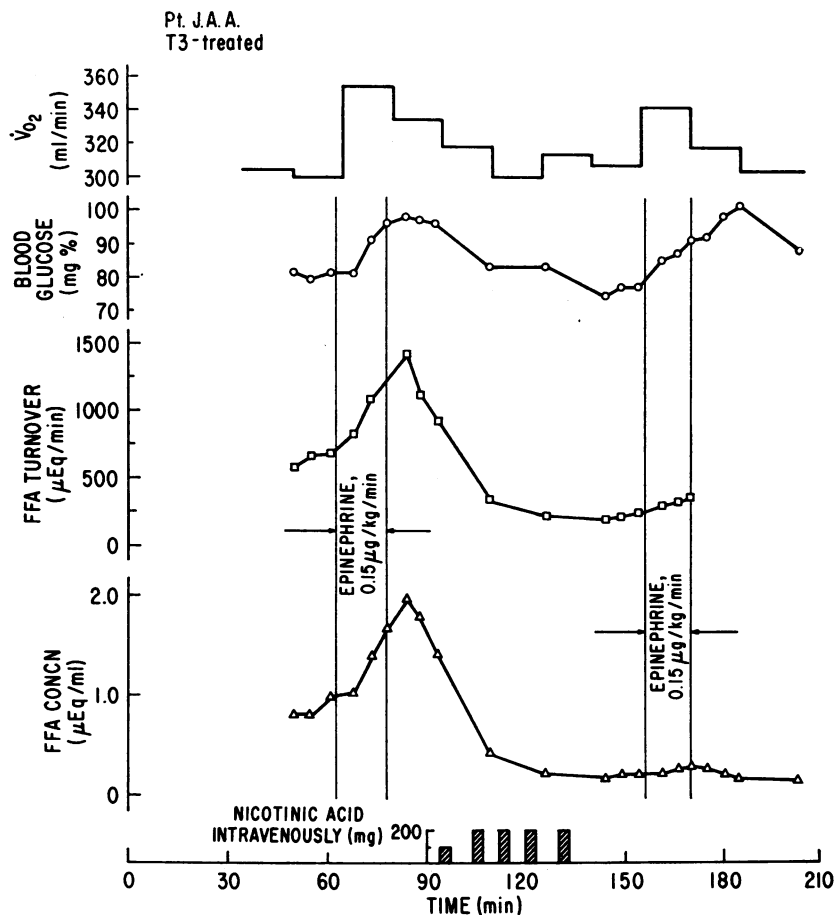


FIG. 7. MODIFICATION BY NICOTINIC ACID OF THE FFA-MOBILIZING AND CALORIGENIC EFFECTS OF EPINEPHRINE. The hyperglycemic effect does not appear to be altered.

to bladder discomfort occasioned by the diuretic effects of the hyperglycemia. Data obtained in subject LC when euthyroid are plotted in Figure 8 and when hyperthyroid in Figure 9. In this study, which was done before the above-mentioned untoward reactions to the drug were reported, pronethalol was given intravenously (100 mg) after the hyperglycemia had been established. It did not appear to alter either FFA concentration or  $\dot{V}O_2$ .

### Discussion

The present studies confirm the results of Carlson, Havel, Ekelund, and Holmgren showing that nicotinic acid profoundly depresses FFA concentration and turnover in normal subjects (13). Since it has been shown to suppress FFA

release from isolated adipose tissue *in vitro* (14, 15), the effect of nicotinic acid may be attributed to a direct local action, but additional indirect effects are not ruled out.

Nicotinic acid all but abolished the FFA-mobilizing effect of infused norepinephrine and also reduced its calorigenic effect. In this latter respect, nicotinic acid was less effective than pronethalol (9). Whereas pronethalol almost completely suppressed the calorigenic response, nicotinic acid reduced it by only about 50%. At the same time, pronethalol had little effect on basal FFA concentration and turnover (9), whereas nicotinic acid caused a marked decrease in both, indicating that the mechanism of action of the two drugs may be different. Havel and co-workers have already observed this suppression

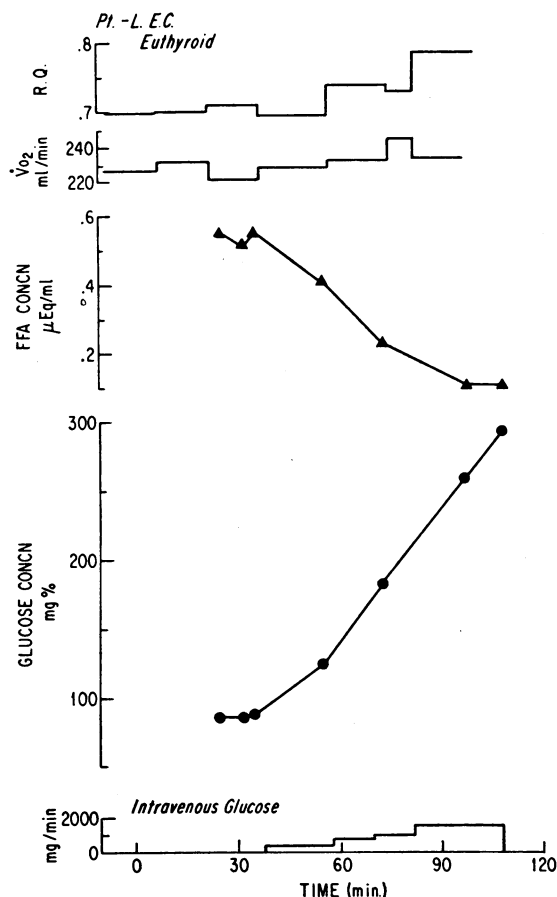


FIG. 8. EFFECTS OF IV GLUCOSE ON RQ, OXYGEN CONSUMPTION, FFA CONCENTRATION, AND GLUCOSE CONCENTRATION IN A EUTHYROID SUBJECT. Data for studies in the same subject when hyperthyroid are shown in Figure 9.

of calorogenic and FFA-mobilizing action (16). The fact that these two effects of norepinephrine are both modified does not, of course, establish a cause and effect relationship between them. The fact that two different blocking agents with apparently different modes of action suppress both effects somewhat strengthens the interpretation that there is a relationship between FFA mobilization and calorogenesis. However, the FFA-mobilizing effect and the calorogenic effect, as pointed out previously (9), may represent two independently determined actions of the catecholamine, both of which are blocked by the agents tested. In fact, the studies reported here, showing that the rate of FFA mobilization can be varied widely without concomitant changes in

oxygen consumption, would appear to argue against a cause and effect relationship.

Treatment with  $T_3$  led to a highly significant increase in basal plasma FFA concentration, consonant with the results of Rich, Bierman, and Schwartz (17) and those of Harlan, Laszlo, Bogdonoff, and Estes (18). The present studies now show that the elevated FFA levels in the hyperthyroid state in man are associated with markedly elevated FFA turnovers. Taken as a whole, the data indicate that the increase in FFA turnover was proportionally greater than the increase in FFA concentrations, although this varied from case to case. The increase in absolute and fractional turnovers may be in part attributable to the increase in total blood flow, which is suggested by the observed increase in cardiac rate (averaging 65 per minute in the euthyroid state, 99 per minute in the hyperthyroid state). However, there is reason to believe that a metabolic effect on triglyceride breakdown or esterification is also involved, since isolated adipose tissue from hyperthyroid animals has been shown to release FFA more rapidly than tissue from normal animals (10, 20). Nicotinic acid very effectively reduced both FFA levels and turnover in the  $T_3$ -treated subjects, in some cases to values below those seen in the euthyroid state. This result is compatible with the suggestion that the high rate of fat mobilization in the hyperthyroid state is secondary to an increase in the sensitivity of adipose tissue to stimulation by catecholamines.

Nicotinic acid was without effect on the elevated rate of oxygen consumption in  $T_3$ -treated subjects, even though it sharply reduced FFA levels and turnover. The same was true when intravenous glucose was used to suppress FFA mobilization. These studies show that maintenance of the elevated metabolic rate in the hyperthyroid state is not dependent upon the high FFA levels and turnover characteristic of it. The findings lend no support to the hypothesis that the high FFA levels found in hyperthyroidism produce and maintain the hypermetabolic state by uncoupling oxidative phosphorylation or by exerting a substrate-concentration effect on rate of oxidation (21, 22). In fact, both in the euthyroid and in the hyperthyroid states, metabolic rate was maintained unchanged in the face

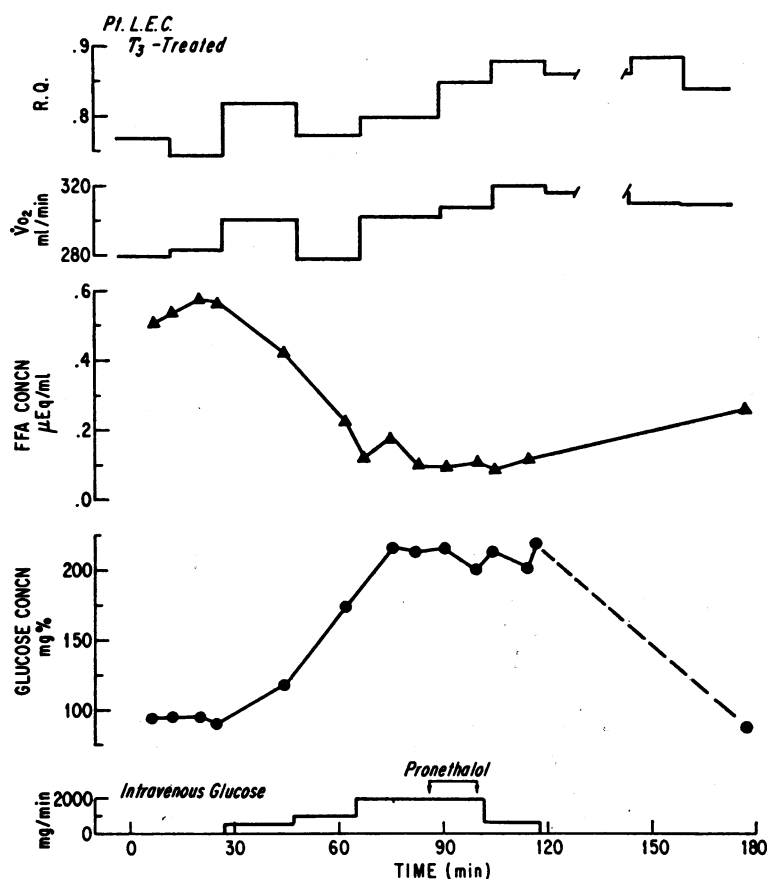


FIG. 9. EFFECTS OF IV GLUCOSE AND OF A SUPERIMPOSED IV DOSE OF PRONETHALOL (100 MG) ON RQ, OXYGEN CONSUMPTION, FFA CONCENTRATION, AND GLUCOSE CONCENTRATION DURING TREATMENT WITH TRIIODOTHYRONINE. Data for same subject studied when euthyroid are shown in Figure 8.

of wide variations in FFA turnover. However, observations in the present studies were limited to acute, short-term periods of interruption of FFA mobilization. It is possible that if the turnover of FFA were maintained at the low levels produced by nicotinic acid treatment for extended periods, an effect on metabolic rate might then become evident.

Whatever the mechanism of the calorogenic action of catecholamines may be, it is counteracted in some fashion by nicotinic acid. Yet in the hyperthyroid subjects, nicotinic acid failed to reduce the elevated metabolic rate, at least acutely. This finding does not lend support to the idea that the hypermetabolism of the hyperthyroid state is attributable to the calorogenic action of catecholamines. The demonstrated ability of nicotinic acid to counteract the effect of exogenously

administered (circulating) catecholamine may not necessarily be matched by an ability to counteract the effects of catecholamine locally released (at nerve endings). Furthermore, this interrelationship has only been studied over a limited range of drug dosages.

A finding of great interest emerging from the present studies is the gross disparity between total calorie consumption and total FFA turnover after administration of nicotinic acid. The RQ showed no consistent change either during or after intravenous administration of nicotinic acid (11 subjects). The mean value during the base-line period was  $0.71 \pm 0.018$  and after nicotinic acid  $0.71 \pm 0.015$ . The RQ was calculated from integration of  $\dot{V}O_2$  and  $\dot{V}CO_2$  data in two successive 15-minute periods immediately following nicotinic acid treatment. The

agreement between the RQ values calculated from these two successive periods was generally good, but in some cases they differed by as much as 0.06 U. Thus small differences due to nicotinic acid might not be detected. Havel and his colleagues (16), on the other hand, have noted a small but significant rise in RQ (from 0.75 to 0.82) due to nicotinic acid under similar conditions. In any case, it is clear that fat continued to be the major, although perhaps not the exclusive, metabolic fuel in the basal period and after nicotinic acid treatment in the present studies. As shown in Table IV, even if all of the FFA being turned over in the plasma compartment were channeled directly or indirectly into the oxidative pathway, this could in some cases account for only one-half or less of the total substrate being utilized by these fasting subjects. Moreover, this disparity was shown to persist over at least 1 hour. In subjects MRF and JAA the difference between total calculated fat oxidized and observed FFA turned over shows that about 5 g of fat from some other source must have been oxidized during the hour of observation. One possibility is that utilization of fatty acid esters carried in the plasma lipoproteins was increased. A second and more likely possibility is that the peripheral tissues draw upon their endogenous stores of lipid to maintain the rate of total substrate oxidation unimpaired even when the minute-to-minute delivery of FFA is acutely interrupted, as in these studies. There is good evidence from *in vitro* studies that the diaphragm and the myocardium can go on respiring for a considerable period of time in the absence of exogenous substrate and that these tissues are utilizing stored lipids (23, 24). Although the turnover of plasma FFA in a true steady state may be well correlated with total energy utilization in the fasting individual, it is clear that when the steady state is interrupted there need not be any such close relationship. Havel, Naimark, and Borchgrevink have noted a similar disparity during exercise and proposed that oxidation of stores of tissue lipid might be of relatively greater importance in working muscle (25). The total lipid content of skeletal muscle in mammals has been reported to be approximately 50 mg per g of wet weight (26). The exact figure for true *intracellular* lipid in muscle is uncertain, but even if it is only 2

or 3 mg per g, the average human subject would have a "reserve" of skeletal muscle lipid of 50 to 100 g. Values for other tissues are of similar magnitude, and so it is clear that the organism as a whole could carry on for some time without depending upon delivery of fatty acid substrate from adipose tissue stores. The concept of a "buffering reservoir" of lipid substrate may be useful. In view of the remarkable rapidity of FFA turnover and its lability, it is obvious that the presence of such a "buffering reservoir" of tissue lipid would be important for calorogenic homeostasis.

The possibility that the therapeutic effectiveness of nicotinic acid in lowering serum lipoprotein levels may be attributable to its ability to suppress FFA mobilization, as suggested by Carlson and Orö (10), is an attractive one. It has been shown that catecholamine administration in several species leads to elevation not only of plasma FFA levels but also of plasma lipoprotein levels, and thus to elevation of cholesterol, phospholipid, and triglyceride levels (27-30).

Studies of isolated perfused rat liver have shown directly that lipoprotein production and secretion are stimulated when the perfusing fluid contains a high concentration of FFA (31-33). If nicotinic acid treatment has the effect of reducing the FFA "load" presented to the liver, this might reduce the lipoprotein output from the liver.

### Summary

The effectiveness of intravenously administered nicotinic acid in blocking almost completely the rise in free fatty acid (FFA) levels and turnover induced by catecholamines was confirmed in normal young adults. It was shown to be effective also in triiodothyronine-treated subjects. The calorogenic response to intravenously administered catecholamines was also reduced, although not completely abolished, by prior administration of nicotinic acid. On the other hand, nicotinic acid did not alter basal metabolic rate or the respiratory quotient either in normal subjects or in triiodothyronine-treated subjects with elevated basal metabolic rate.

Administration of glucose to control and to triiodothyronine-treated subjects reduced FFA levels to very low values, but again did not reduce

metabolic rate. Although the hyperthyroid state is associated with elevated FFA levels and turnover, the present results demonstrate that the hypermetabolism persists unaltered when FFA levels and turnover are reduced, at least for short time periods, by administration of nicotinic acid or glucose.

Under the influence of nicotinic acid, there was a large disparity between total calorie consumption and total FFA turnover. We suggest that stored tissue lipids provide a "buffering reservoir" of substrate in this and similar situations, i.e., that the tissues call on endogenous substrate to support respiration at an unimpaired rate for some time, even though the rate of delivery of FFA from the depots is temporarily reduced to low values.

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