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### Research Article

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# Lipolysis during Fasting

## Decreased Suppression by Insulin and Increased Stimulation by Epinephrine

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### Abstract

These studies were designed to determine whether the insulin resistance of fasting extends to its antilipolytic effects and whether fasting enhances the lipolytic effects of adrenergic stimulation independent of changes in plasma hormone and substrate concentrations. Palmitate flux was determined isotopically ( $[1-^{14}\text{C}]$ palmitate) before and during epinephrine infusion in normal volunteers after a 14-h (day 1) and an 84-h (day 4) fast. Using a pancreatic clamp, constant plasma hormone and glucose concentrations were achieved on both study days in seven subjects. Six subjects were infused with saline and served as controls. During the pancreatic clamp, palmitate flux was greater ( $P < 0.01$ ) on day 4 than day 1, despite similar plasma insulin, glucagon, growth hormone, cortisol, epinephrine, norepinephrine, and glucose concentrations. The lipolytic response to epinephrine was greater ( $P < 0.05$ ) on day 4 than day 1 in both groups of subjects. In conclusion, lipolysis during fasting is less completely suppressed by insulin and more readily stimulated by epinephrine.

### Introduction

The orderly, increased release of free fatty acids (FFA) into the circulation during fasting provides an important alternative fuel to glucose for most body tissues. It is likely that the decrease in plasma insulin concentration that occurs during fasting is involved in regulating lipolysis, since insulin is a potent antilipolytic hormone, even at relatively low plasma concentrations (1). In addition, decreased adipose tissue sensitivity to insulin could play a role. Although fasting is known to induce resistance to insulin's ability to stimulate glucose utilization (2), it remains controversial whether its antilipolytic properties are also affected (3–5).

In addition to insulin, adrenergic mechanisms regulate FFA availability (6). In human adipocytes,  $\beta_1$ -adrenergic receptors mediate stimulation of lipolysis by catecholamines and  $\alpha_2$ -adrenergic receptors mediate inhibition of lipolysis (7–9).  $\beta$ -adrenergic mechanisms appear to predominate both in vitro (8) and in vivo; infusions of mixed  $\alpha$ - and  $\beta$ -adrenergic agonists such as epinephrine result in increased lipolysis as evidenced by increments in the circulating levels of FFA and glycerol and

increments in FFA appearance rates (6). Fasting has been reported to increase adipocyte  $\beta$ -adrenergic receptor numbers in rats (10) but to decrease  $\beta$ -adrenergic receptor number in some human adipose tissue sites (11). Adipocytes from calorically restricted or fasting humans exhibit increased basal lipolytic rates but decreased lipolytic (indeed even antilipolytic) responses to epinephrine and norepinephrine (11–16). The increase in basal lipolysis and reversal of the effect of epinephrine can be mimicked in vitro by removal of endogenous adenosine (16), suggesting that the changes associated with fasting are the result of decreased inhibition of lipolysis by local modulators (16, 17). Clearly, these in vitro findings do not explain the mechanisms of increased basal and catecholamine-stimulated lipolysis (18–21) in vivo during fasting. Lower ambient insulin secretion could explain both increased basal and catecholamine-stimulated lipolysis (22). In addition, increments in lipolytic hormones, such as growth hormone (23) and glucagon (24), as well as the catecholamines, could explain increased lipolysis, since their plasma concentrations are increased during fasting (25). If these hormonal adaptations are solely responsible for increased lipolysis during fasting, the latter should be reversed if the hormone concentrations were adjusted experimentally to pre-fast levels during fasting.

The following studies were undertaken to test the hypotheses that fasting in normal humans both reduces insulin's antilipolytic potency and enhances the lipolytic effect of epinephrine in vivo when plasma glucose, insulin, glucagon, growth hormone, epinephrine, and norepinephrine concentrations are identical.

### Methods

**Subjects.** Informed written consent was obtained from 13 normal, healthy volunteers (age  $27 \pm 2$  yr, mean  $\pm$  SEM, body mass index  $21.2 \pm 0.5$  kg/m<sup>2</sup>). The subjects were taking no medications, had no family history of diabetes, and had maintained a stable weight for at least 2 mo before this study. All subjects consumed a diet containing  $>200$  g of carbohydrate daily for at least 2 wk before this study.

**Materials.**  $[1-^{14}\text{C}]$ Palmitate (Research Products Int. Corp. Mt. Prospect, IL) was prepared for intravenous infusion by evaporating the solution to dryness under nitrogen and resuspending the palmitate in 0.9% NaCl containing 5% albumin (25% normal serum albumin; Travenol Laboratories, Glendale, CA). Aliquots (1.5 cm<sup>3</sup>,  $\sim 150$   $\mu\text{Ci}$ ) were transferred directly into sterile single-dose 5-cm<sup>3</sup> vials through a Millipore filter (Acrodisc, Gelman Sciences, Inc., Ann Arbor, MI). Samples were demonstrated to be sterile and pyrogen free. On the day of the study,  $\sim 150$   $\mu\text{Ci}$  of the  $[1-^{14}\text{C}]$ palmitate-albumin solution was suspended in 59 cm<sup>3</sup> of sterile, nonbacteriostatic NaCl and infused as described below. Insulin (Actrapid Human; Squibb-Novo, Inc., Princeton, NJ), glucagon (Eli Lilly & Co., Indianapolis, IN), human growth hormone (National Pituitary Agency, Baltimore, MD), somatostatin (courtesy of Drs. Roger Guillemin and Nicholas Ling, Salk Institute, San Diego, CA), epinephrine (Parke, Davis & Co., Detroit, MI), and ascorbic acid (Cevalin; Eli Lilly & Co.) were used in this study.

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**Protocols.** Subjects were admitted to the Mayo Clinic Clinical Research Center the evening before the first study and given a standard meal. All subjects were then studied on two occasions in the postabsorptive state, after 14 h (day 1) and again after 84 h (day 4) of fasting. Two different experimental protocols were used; the experimental protocol performed on each individual was identical on day 1 and day 4. The design is outlined in Fig. 1. The subjects remained hospitalized in the Clinical Research Center through completion of the study, where they had free access to water but were not allowed to eat. Urinary ketones were measured 4 to 6 times daily to monitor compliance with the fast.

On the morning of each study, a 19-gauge scalp vein needle was inserted into a dorsal hand vein in a retrograde fashion and the hand was placed in a heated (50°–55°C) box for sampling of arterialized blood as previously described (26). An 18-gauge infusion catheter was placed in a forearm vein in the contralateral arm and kept patent by a controlled infusion of 0.9% NaCl (20 ml/h). After the placement of catheters, a primed (30  $\mu$ Ci) continuous (0.3  $\mu$ Ci/min) infusion of [1- $^{14}$ C]palmitate was begun at –210 min (0700 h) via a Harvard Pump and continued through +270 in all subjects. Infusion rates of [1- $^{14}$ C]palmitate were  $5,228 \pm 544$  dpm  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$  for clamp subjects and  $6,270 \pm 753$  dpm  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$  for control subjects (see below).

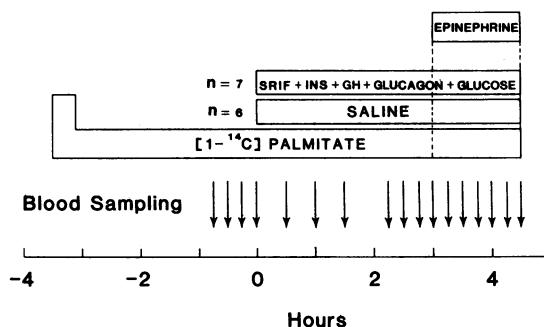
In one group of subjects ( $n = 7$ ), two additional 18-gauge catheters were placed in separate forearm veins and used for continuous blood withdrawal by a Biostator (Life Science Instruments, Miles Laboratory, Elkhart, IN) and used solely for constant glucose monitoring (27). A “pancreatic clamp” was begun at minute 0 (somatostatin, 200  $\mu$ g/h; growth hormone, 4 ng/kg $^{-1}$ /min $^{-1}$ ; glucagon, 0.6 ng/kg $^{-1}$ /min $^{-1}$ ; and insulin, 0.20 mU/kg $^{-1}$ /min $^{-1}$ ). (These studies were completed before the announcement of potential viral contamination of National Pituitary Agency human growth hormone). The hormones were suspended in 0.9% NaCl containing 1% human serum albumin and infused via a Harvard Pump. An intravenous bolus of 0.14 mU/kg of insulin was given at minute 0 and variable amounts of glucose infused with a Harvard pump to maintain the plasma glucose concentration constant at 85 mg/dl in all subjects, as previously described (27). Because the plasma glucose concentrations were appreciably lower after 84 h of fasting, increased amounts of glucose were infused during the first 15–30 min of the pancreatic clamp on day 4 to increase the plasma glucose concentrations to 85 mg/dl. Blood was sampled from the pancreatic clamp subjects at 15-min intervals from minute 0 to +270 and assayed immediately for plasma glucose concentration. From minute 180 to 270 minutes, subjects were infused with epinephrine-diluted 0.9% saline containing ascorbic acid (1 mg/ml) at a rate of 13 ng/kg $^{-1}$ /min $^{-1}$ . In the second group of experiments, six subjects were studied in an identical fashion except they received an infusion of saline in place of the pancreatic clamp and served as controls.

Blood was sampled before starting the isotope infusion on each day and assayed for palmitate specific activity (sp act)<sup>1</sup> to serve as background for that day. Blood samples were obtained at –210, –45, –30, –15, 0, +30, +60, +90, and +135 min, and every 15 min thereafter, and analyzed for plasma concentrations of total FFA (28), glucose (YSI Glucose Analyzer; Yellow Springs Instruments, Yellow Springs, OH), beta-hydroxybutyrate and acetoacetate (3), and palmitate concentrations and sp act (29). Plasma insulin (30), C-peptide (31), glucagon (32), growth hormone (33), epinephrine, and norepinephrine (34) concentrations were determined at –30, –15, 0, +150, +165, +180, +240, +255, +270 min.

Plasma palmitate sp act and concentration were determined by a high performance liquid chromatography technique recently developed in our laboratory (29), with the exception that [9,10- $^3$ H]palmitate was used as an internal standard. The coefficient of variation for replicate analysis using this procedure is 3.8% for concentration and 2.3% for specific activity.

### Calculations

Minute –45 to 0 will be referred to as the “baseline” study period; minute 135 to 180 as the “clamp” study period for subjects undergoing the



**Figure 1.** Design of the control and pancreatic clamp studies. The experimental protocol performed on each subject was identical after an overnight fast and an 84-h fast. See Methods section of text for description. SRIF, somatostatin; INS, insulin; and GH, human growth hormone.

pancreatic clamp, and “saline” study period for control subjects; and minute 195 to 270 as the “epinephrine” study period. Since plasma hormone concentrations were stable during each study period, the average values for each study period were determined for each subject and used to calculate the group mean and standard error.

Plasma palmitate concentrations and specific activities were nearly constant during the baseline study period and the third hour of the clamp or saline study periods; therefore, steady state conditions were assumed. Palmitate flux was calculated for each individual during these study periods using mean specific activity and the standard formula:  $F[^{14}\text{C}]\text{palmitate}/\text{SA}[^{14}\text{C}]\text{palmitate}$ , where  $F[^{14}\text{C}]\text{palmitate}$  is the rate of isotope infusion (dpm  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ ) and  $\text{SA}[^{14}\text{C}]\text{palmitate}$  is the specific activity of [ $^{14}\text{C}$ ]palmitate in plasma.

The same equation was used to calculate FFA turnover, except the total FFA sp act was substituted for sp act [ $^{14}\text{C}$ ]palmitate. The sp act for plasma total FFA was calculated, using palmitate as a paradigm for all plasma FFAs, from the following formula:  $[(\text{palmitate SA})(\text{palmitate concentration})]/(\text{total FFA concentration})$ . Plasma palmitate concentrations were consistently 20–23% of total FFA concentrations over the entire range of FFA concentrations observed (60–3,500  $\mu$ M), suggesting that palmitate flux measurements provide reasonable fractional estimates of total FFA flux. In this report, therefore, FFA turnover data is expressed almost exclusively as palmitate flux.

During the epinephrine infusion, palmitate sp act and concentrations were not constant. Recent studies performed in our laboratory (29) have shown that when FFA flux is changing, the best estimate of rate of appearance at a given sampling time is provided by the steady state equations described above. Therefore, the lipolytic response to epinephrine was assessed by calculating the palmitate rate of appearance at 15-min intervals during the epinephrine infusion, and determining both the area under the curve of palmitate rate of appearance above baseline and the peak increment in palmitate rate of appearance. Because of blood volume sampling limitations, palmitate sp act and concentration were not determined at minute 195 in clamp subjects.

All results are expressed as mean  $\pm$  SE. Statistical comparisons between the same study periods on day 1 vs. day 4 in the same group were determined using a two-tailed paired Student's *t* test, as were comparisons between one time interval and another on the same study day. Comparisons between groups at the same time intervals were made using a two-tailed nonpaired Student's *t* test.

## Results

**Baseline hormone and substrate concentrations, FFA, and palmitate flux (Table I).** As expected, after 84 h of fasting, plasma concentrations of glucose decreased ( $P < 0.001$ ) while those of palmitate, total FFA, and ketone bodies increased (all  $P < 0.001$ ). Plasma concentrations of insulin were lower on day 4 than day 1

1. Abbreviation used in this paper: sp act, specific activity.

Table I. Baseline Plasma Hormone and Substrate Concentrations in All Subjects (n = 13)

	Day 1	Day 4
Insulin ( $\mu\text{U/ml}$ )	6.6 $\pm$ 0.4	4.2 $\pm$ 0.2*
C-peptide ( $\text{ng/ml}$ )	0.9 $\pm$ 0.08	0.3 $\pm$ 0.02*
Glucagon ( $\text{pg/ml}$ )	192 $\pm$ 14	345 $\pm$ 20*
Growth hormone ( $\text{ng/ml}$ )	2.8 $\pm$ 0.6	3.7 $\pm$ 0.5
Cortisol ( $\mu\text{g/dl}$ )	22 $\pm$ 2	25 $\pm$ 2
Epinephrine ( $\text{pg/ml}$ )	49 $\pm$ 7	74 $\pm$ 8 <sup>‡</sup>
Norepinephrine ( $\text{pg/ml}$ )	193 $\pm$ 15	247 $\pm$ 18 <sup>‡</sup>
Glucose ( $\text{mg/dl}$ )	94 $\pm$ 1	62 $\pm$ 2*
Free fatty acids ( $\mu\text{M}$ )	565 $\pm$ 62	1,475 $\pm$ 82*
Palmitate ( $\mu\text{M}$ )	130 $\pm$ 13	321 $\pm$ 15*
Acetoacetate ( $\mu\text{M}$ )	100 $\pm$ 20	1,230 $\pm$ 80*
$\beta$ -Hydroxybutyrate ( $\mu\text{M}$ )	240 $\pm$ 70	5,030 $\pm$ 400*

Different than day 1: \* $P < 0.001$ . <sup>‡</sup> $P = 0.01$ . <sup>§</sup> $P < 0.05$ . Overnight (14 h) fast, day 1; 84-h fast, day 4. All data expressed as mean $\pm$ SE.

( $P < 0.001$ ); however, the magnitude of the decline in insulin secretion is better reflected by the plasma C-peptide concentrations, which were 70% lower on day 4. Plasma glucagon concentrations were higher ( $P < 0.001$ ) after brief fasting, while the increase in growth hormone concentrations was not significant. Baseline plasma concentrations of epinephrine and norepinephrine were significantly higher after fasting. Baseline palmitate and FFA fluxes were  $1.35 \pm 0.35$  and  $6.19 \pm 0.97 \mu\text{mol/kg}^{-1}/\text{min}^{-1}$ , respectively, on day 1 and increased to  $2.65 \pm 0.15$  and  $12.18 \pm 0.9$ , respectively, on day 4 ( $P < 0.001$ ).

**Hormone values—baseline to saline or clamp (Table II).** In the control group, plasma concentrations of insulin, C-peptide, glucagon, and growth hormone did not change significantly between baseline and saline study periods on either day 1 or day 4. During the pancreatic clamp, the chosen insulin infusion rate resulted in plasma insulin concentrations of  $\sim 12 \mu\text{U/ml}$  on both study days, which were not significantly different from each other. Plasma concentrations of growth hormone and glucagon did not change from basal values when the pancreatic clamp was instituted on day 1, and were similar during the clamp periods on days 1 and 4.

**Plasma catecholamine concentrations (Table II).** On days 1 and 4, plasma epinephrine and norepinephrine concentrations increased slightly, but not significantly, between baseline and saline study periods in the control subjects; a similar trend was noted on day 1 between baseline and clamp study periods in subjects undergoing the pancreatic clamp. However, during the day-4 pancreatic clamp, plasma epinephrine and norepinephrine concentrations decreased to values not significantly different from those observed during the day-1 clamp study period ( $56 \pm 8$  vs.  $88 \pm 23 \text{ pg/ml}$  and  $234 \pm 17$  vs.  $229 \pm 26 \text{ pg/ml}$ , respectively). The decline in plasma norepinephrine concentrations was statistically significant. During the intravenous epinephrine infusions, plasma epinephrine and norepinephrine concentrations were not different between groups or between study days.

**FFA response to clamp and saline.** When plasma insulin concentrations were increased, but plasma glucagon and growth hormone concentrations maintained constant during the pancreatic clamp on day 1, plasma concentrations of ketone bodies (data not shown), total FFA, and palmitate (Fig. 2) decreased. Palmitate sp act increased (Fig. 2) and remained stable during

the third hour of the pancreatic clamp. On day 4 the same pattern of changes was observed (Fig. 2); however, plasma palmitate concentration and flux during the third hour of the clamp remained higher ( $P < 0.01$ ) on day 4 than day 1 ( $111 \pm 14$  vs.  $34 \pm 8 \mu\text{M}$  and  $1.37 \pm 0.17$  vs.  $0.68 \pm 0.11 \mu\text{mol/kg}^{-1}/\text{min}^{-1}$ , respectively). In saline-infused control subjects, palmitate flux increased slightly, but not significantly, from the baseline to the saline study period on day 1 ( $1.15 \pm 0.13$  vs.  $1.54 \pm 0.58 \mu\text{mol/kg}^{-1}/\text{min}^{-1}$ ), while on day 4 palmitate flux was stable between the baseline and saline study periods ( $2.42 \pm 0.24$  vs.  $2.47 \pm 0.28 \mu\text{mol/kg}^{-1}/\text{min}^{-1}$ ).

**Glucose and hormone responses to epinephrine infusion.** During the 90-min epinephrine infusion in subjects undergoing the pancreatic clamp, no changes occurred in plasma concentrations of glucose, insulin, C-peptide, glucagon, or growth hormone relative to the clamp study period on either day 1 or day 4. On day 1, in the saline-infused control subjects, epinephrine infusion resulted in increased plasma concentrations of glucose ( $93 \pm 3$ – $106 \pm 5$ ,  $P < 0.01$ ) and insulin ( $P < 0.005$ ) (Table II), while those of glucagon and growth hormone did not change significantly. On day 4, plasma glucose concentrations increased ( $P < 0.05$ ) from  $61 \pm 2$  to  $67 \pm 3 \text{ mg/dl}$ , as did those of insulin ( $P < 0.005$ ) and glucagon ( $P < 0.05$ ), while that of growth hormone decreased ( $P < 0.05$ ) (Table II).

**FFA responses to epinephrine infusion.** During the epinephrine infusion, plasma palmitate and total FFA concentrations increased, palmitate specific activity decreased, and palmitate rate of appearance increased in all subjects (Figs. 3 and 4). In control subjects, a greater ( $P < 0.05$ ) lipolytic response to epinephrine was observed on day 4 than day 1, whether measured as area under the curve above baseline palmitate rate of appearance ( $147 \pm 23$  vs.  $92 \pm 15 \mu\text{mol/kg}$ ) or peak increment of palmitate rate of appearance ( $2.6 \pm 0.3$  vs.  $1.5 \pm 0.3 \mu\text{mol/kg}^{-1}/\text{min}^{-1}$ ). A greater lipolytic response to epinephrine was observed in subjects undergoing the pancreatic clamp on day 4 than day 1 by area under the curve ( $109 \pm 19$  vs.  $57 \pm 15 \mu\text{mol/kg}$ ,  $P < 0.05$ ) and peak increment in palmitate rate of appearance ( $2.2 \pm 0.3$  vs.  $0.9 \pm 0.2 \mu\text{mol/kg}^{-1}/\text{min}^{-1}$ ,  $P < 0.005$ ). These values should be considered minimum estimates for both study days because of the long sampling interval relative to the half-life of circulating FFA.

**Heart rate response to epinephrine infusion.** No subjective responses to epinephrine were reported by any of the subjects on day 1 of the study, and little change in heart rate was observed (an increase of  $5 \pm 1$  beats/min). On day 4, however, 9 of 11 subjects reported symptoms of adrenergic stimulation, including forceful heart beat, tremulousness, and nervousness. They were also found to have greater increases in heart rate ( $\Delta = +15 \pm 3$  beats/min,  $P < 0.05$  vs. day 1). These symptoms always occurred between 15 and 45 min after beginning the epinephrine infusion and were resolved within 15 to 30 min despite continued infusion of epinephrine. In two subjects, plasma catecholamine concentrations were measured 15 and 30 min after beginning the epinephrine infusion on both study days, to ensure that they were not higher during the symptomatic time interval; the values were identical to those observed between 60 and 90 min of the epinephrine infusion (data not shown).

## Discussion

The purpose of these studies was to determine whether fasting in normal humans results in resistance to insulin's antilipolytic

Table II. Plasma Hormone Concentrations\*

Pancreatic clamp subjects (n = 7)						
	Day 1			Day 4		
	Baseline	Clamp	EPI‡	Baseline	Clamp	EPI
Insulin ( $\mu\text{U/ml}$ )	5.7 $\pm$ 0.4	12.3 $\pm$ 0.2	12.4 $\pm$ 0.3	4.1 $\pm$ 3	11.6 $\pm$ 0.4	12.0 $\pm$ 0.4
Glucagon (pg/ml)	193 $\pm$ 23	194 $\pm$ 20	195 $\pm$ 19	323 $\pm$ 32	202 $\pm$ 24	190 $\pm$ 23
C-peptide (ng/ml)	0.76 $\pm$ 0.09	0.14 $\pm$ 0.01	0.13 $\pm$ 0.01	0.29 $\pm$ 0.02	0.13 $\pm$ 0.01	0.12 $\pm$ 0.01
Growth hormone (ng/ml)	2.6 $\pm$ 0.3	2.3 $\pm$ 0.5	1.8 $\pm$ 0.1	3.3 $\pm$ 0.5	2.7 $\pm$ 0.4	2.0 $\pm$ 0.2
Epinephrine (pg/ml)	64 $\pm$ 9	88 $\pm$ 23	323 $\pm$ 16	82 $\pm$ 12	56 $\pm$ 8	287 $\pm$ 16
Norepinephrine (pg/ml)	203 $\pm$ 21	229 $\pm$ 26	250 $\pm$ 16	288 $\pm$ 18	234 $\pm$ 17 <sup>†</sup>	283 $\pm$ 6
Control subjects (n = 6)						
	Day 1			Day 4		
	Baseline	Saline	EPI	Baseline	Saline	EPI
Insulin ( $\mu\text{U/ml}$ )	7.6 $\pm$ 0.6	7.0 $\pm$ 0.8	9.1 $\pm$ 1.0	4.4 $\pm$ 0.4	4.4 $\pm$ 0.6 <sup>§</sup>	6.6 $\pm$ 0.6 <sup>§  </sup>
Glucagon (pg/ml)	190 $\pm$ 16	192 $\pm$ 19	211 $\pm$ 14	371 $\pm$ 22	368 $\pm$ 16 <sup>§</sup>	413 $\pm$ 22 <sup>§†</sup>
C-peptide (ng/ml)	1.1 $\pm$ 0.1	0.86 $\pm$ 0.1	1.1 $\pm$ 0.1	0.31 $\pm$ 0.03	0.27 $\pm$ 0.04 <sup>§</sup>	0.58 $\pm$ 0.09 <sup>§  </sup>
Growth hormone (ng/ml)	3.0 $\pm$ 1.3	5.8 $\pm$ 1.5	2.1 $\pm$ 0.3	4.2 $\pm$ 1.1	4.4 $\pm$ 0.8	2 $\pm$ 0.2 <sup>†</sup>
Epinephrine (pg/ml)	31 $\pm$ 6	47 $\pm$ 8	287 $\pm$ 20	64 $\pm$ 7	73 $\pm$ 7	257 $\pm$ 9
Norepinephrine (pg/ml)	181 $\pm$ 21	189 $\pm$ 25	272 $\pm$ 32	198 $\pm$ 17	209 $\pm$ 16	268 $\pm$ 29

\* Baseline values after an overnight fast (day 1) vs. an 84-h fast (day 4) for all subjects were analyzed in Table I; these values are not reanalyzed here. Because insulin, glucagon, growth hormone, and C-peptide values are not random variables in pancreatic clamp subjects and catecholamine values are not random variables during epinephrine infusion, these values are not analyzed vs. preceding study period same day. All data expressed as mean $\pm$ SE. ‡ EPI, epinephrine infusion study period. §  $P < 0.005$  cf. same study period day 1. ||  $P < 0.005$  cf. prior study period same day. †  $P < 0.05$  cf. prior study period same day.

properties and an enhanced lipolytic response to adrenergic stimulation if plasma concentrations of insulin, glucose, glucagon, and growth hormone are returned to prefasting values. To

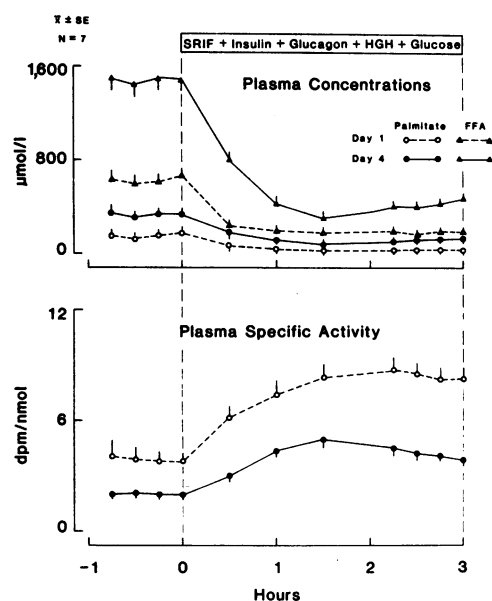


Figure 2. Changes in plasma FFA and palmitate concentration and palmitate specific activity in response to the infusion of somatostatin (SRIF), insulin, glucagon, human growth hormone (HGH), and glucose (a pancreatic clamp) after an overnight fast (day 1-dashed lines) and an 84-h fast (day 4-solid lines).

this end, palmitate flux was measured before and during epinephrine infusion in two groups of subjects after 14 and 84 h of fasting. One group of subjects was studied using the pancreatic clamp technique, while the other group received an intravenous saline infusion and served as controls. As expected, palmitate flux was higher after an 84-h fast than after a 14-h fast. When the plasma insulin and glucose (as well as glucagon and growth hormone) concentrations were rendered constant and identical on both study days, palmitate flux was greater in the 84-h fasted

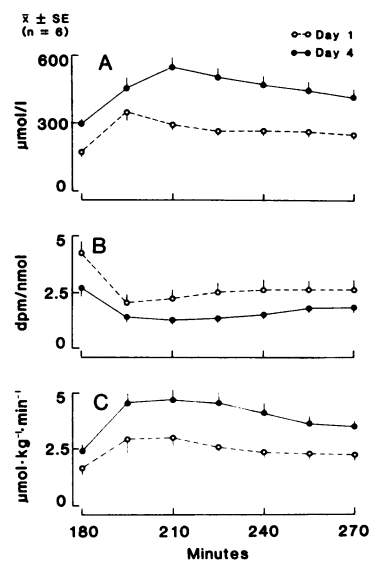


Figure 3. Response of (A) palmitate concentration, (B) specific activity, and (C) rate of appearance in the saline-infused control group to epinephrine infusion (minutes 180-270) after an overnight fast (day 1-dashed lines) and an 84-h fast (day 4-solid lines).

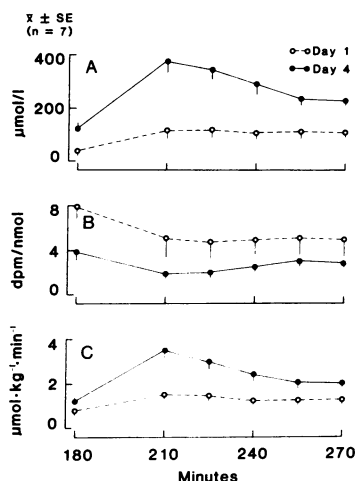


Figure 4. Response of (A) palmitate concentration, (B) specific activity, and (C) rate of appearance in the pancreatic clamp group to epinephrine infusion (minutes 180–270) after an overnight fast (day 1-dashed lines) and an 84-h fast (day 4-solid lines).

subjects. These data provide strong evidence of fasting-induced insulin resistance with regards to lipolysis. Intravenous epinephrine infusion after 84 h of fasting resulted in greater increases in palmitate flux than after 14 h of fasting in both the saline-infused and pancreatic clamp subjects. Therefore, the fasting-enhanced lipolytic response to epinephrine infusion cannot be accounted for solely by changes in plasma glucose and hormone concentrations.

The finding of fasting-induced insulin resistance with regards to lipolysis is consistent with the conclusions of Cahill et al. (3), but at variance with those of two more recent studies (4, 5) involving insulin administration to humans before and after fasting. In these two studies (4, 5), the antilipolytic activity of insulin was assessed as the decrease in plasma FFA concentrations from baseline, whereas the present study compares the absolute FFA flux at a given insulin concentration before and after fasting. The latter approach was selected because of the vastly differing hormone and substrate concentrations before and after fasting, and because of the inherent difficulties in comparing changes from baseline (35). The finding that greater amounts of circulating FFA are present after fasting under conditions of identical insulin concentrations supports the concept of fasting-induced insulin resistance with regards to lipolysis.

This conclusion also appears to be at variance with *in vitro* studies suggesting that fasting enhances insulin's antilipolytic properties (36–40), perhaps via increased high affinity adipocyte insulin receptor binding (38–40). The study of obese subjects (36–40), a longer duration of fasting (36–40), and regional differences in the response of adipocytes to various lipolytic and antilipolytic stimuli (15, 36, 37, 40) may provide partial explanations for the apparent discrepancy between those studies and the present report. The most likely explanation for the different conclusions, however, are differences in data interpretation and perspective. *In vitro* studies have demonstrated marked increases in basal lipolysis after fasting and define insulin responsiveness as the decrease in glycerol release from basal values, either in relative or absolute terms (39, 40). The amount of glycerol released at the maximally suppressive insulin concentration was greater after fasting, even though the decrement from basal was greater (39, 40). This observation is consistent with greater FFA availability *in vivo*. The increased amounts of circulating FFA may contribute to the observed fasting-induced insulin resistance with regards to glucose utilization (2) via the glucose–fatty acid cycle (41).

Although catecholamines have been reported to stimulate lipolysis to a greater extent after caloric deprivation (19–21), it has not been clear whether this phenomenon is related to changes in adrenergic sensitivity or whether it is mediated indirectly, via changes in plasma concentrations of hormones and substrates known to affect lipolysis. Lower plasma insulin (22) and glucose (42) concentrations, coupled with higher plasma glucagon concentrations (24), might enhance the lipolytic response to catecholamines after fasting. Moreover, epinephrine infusion in overnight fasted control subjects increased plasma concentrations of insulin and glucose, which may restrain lipolysis. The blunted glucose and insulin responses to epinephrine after an 84-h fast may have contributed to the enhanced lipolytic response. However, the lipolytic effect of epinephrine in the subjects undergoing the pancreatic clamp was also greater after fasting, suggesting that this enhanced response is mediated by factors in addition to differences in the measured hormone and substrate concentrations. *In vitro* studies have suggested that fasting increases adipocyte  $\beta$ -adrenergic receptor number (10), thus providing a potential mechanism explaining these observations. The changes in adrenergic sensitivity may not be limited to lipolysis, since the subjects in the present study experienced enhanced cardiovascular responses to epinephrine after fasting.

Note that some studies of catecholamine action on adipose tissue obtained from fasting humans have found changes different from those seen in the present study (12–14). While  $\alpha$ -adrenergic stimulation normally suppresses lipolysis in human adipocytes (12), this effect became dominant in adipocytes from fasting obese individuals (12–14). Increased  $\beta$ -adrenergic responsiveness has also been observed (13). The findings of the present study suggest that in fasting nonobese individuals the net change in  $\alpha$ - and  $\beta$ -adrenergic effects is in favor of increased  $\beta$ -adrenergic responsiveness. Possible explanations for the different conclusions include the relatively greater amounts of catecholamines used in the *in vitro* studies, regional differences in adipocyte response to catecholamines, and the study of obese, as opposed to nonobese, subjects.

Plasma epinephrine and norepinephrine concentrations were higher in our subjects on day 4 of fasting than on day 1, consistent with the findings of others (43, 44). Sodium depletion has been purported to account for the higher plasma catecholamine concentrations observed after fasting (43), since plasma catecholamine concentrations and urinary catecholamine excretion actually decline in sodium-replete subjects during caloric restriction (45). Fasting has been shown to reduce sympathetic neural activity in rodents (44). Thus, fasting-associated increments in plasma catecholamine concentrations are most likely of adrenomedullary origin. When the plasma glucose concentrations in fasting subjects were raised to prefasting values, plasma epinephrine and norepinephrine concentrations decreased to values comparable to those observed on day 1. These observations are more consistent with the hypothesis that the elevated plasma catecholamine concentrations seen with fasting represent an adrenomedullary response to a decrement in plasma glucose concentrations (44).

The enhanced lipolytic and chronotropic responses to epinephrine after fasting were also somewhat surprising, considering that an increase in plasma hormone concentrations (in this case, catecholamines) usually results in down-regulation of receptors (46). If, however, autonomic nervous system activity decreases during fasting (44), up-regulation of postsynaptic adrenergic re-

ceptors may actually occur, which might then allow greater stimulation by the epinephrine administered during this study.

It is conceivable that fasting may alter the clearance of insulin (19), glucagon (47), growth hormone, or epinephrine. However, when endogenous release of insulin, glucagon, and growth hormone was inhibited with somatostatin and these hormones infused on a body weight basis, their plasma concentrations did not differ, indicating that the clearance of these hormones was not significantly altered by fasting. Since infusion of epinephrine may have resulted in differing suppression of endogenous epinephrine release, an equally firm conclusion cannot be reached concerning epinephrine clearance during fasting. However, the increment in plasma epinephrine concentrations was virtually identical on days 1 and 4, suggesting that no change in epinephrine clearance occurred.

In summary, the present studies demonstrate that the accelerated lipolysis of short-term fasting is less completely suppressed by an identical insulin infusion, even when plasma concentrations of glucose, glucagon, and growth hormone (as well as epinephrine) are adjusted to levels comparable to prefasting values. In addition, lipolysis during fasting is more readily stimulated by epinephrine, an observation that cannot be attributed solely to changes in plasma hormone and substrate concentrations. Increased adipocyte sensitivity to  $\beta$ -adrenergic stimulation, in concert with decrements in insulin and increments in epinephrine concentrations, may facilitate FFA mobilization during fasting.

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