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Beta-Blockade Lowers Peripheral Lipolysis in Burn Patients Receiving Growth Hormone

Rate of Hepatic Very Low Density Lipoprotein Triglyceride Secretion Remains Unchanged

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Objective
The purpose of this study was to determine the effect of propranolol on peripheral lipolysis in massively burned children during treatment with recombinant human growth hormone (rhGH), and to ascertain whether decreased free fatty acid availability for re-esterification would alter the hepatic rate of secretion of triglycerides (TGs) bound to very low density lipoproteins (VLDLs).

Background
Fatty liver occurs in severely burned patients, often resulting in a twofold increase in liver size. This could be the result of an imbalance between increased provision of free fatty acids from peripheral lipolysis, coupled with no increase in fat oxidation, and insufficient rate of secretion of TGs from the liver.

Methods
In a cross-over study, six burned children were treated with either rhGH or rhGH plus propranolol. On the sixth day of treatment, isotopic tracer infusions were conducted to determine the rate of release of free fatty acid (Ra FFA) from peripheral tissue and the rate of secretion of VLDL-bound TGs by the liver.

Results
Exogenous rhGH increased Ra FFA in children with large third-degree burns. Propranolol decreased Ra FFA, but the rate of secretion of fatty acids in the form of VLDL-TG from the liver was maintained. Plasma FFA, as opposed to fatty acids newly synthesized in the liver, were the primary precursors for hepatic triglyceride synthesis.

Conclusions
The administration of propranolol to burned children receiving rhGH is safe, has salutary cardiovascular effects, decreases the release of FFA from adipose tissue and increases the efficiency of the liver in secreting fatty acids as VLDL TGs.

Severe burn injury is characterized by both hyperdynamic cardiovascular and metabolic responses. The metabolic response can lead to severe muscle wasting[1,2], poor wound healing, and compromised immunologic function.[3,4] Fat deposition in the liver also is a common clinical finding.[3,5–8] This could be at least partially due to a high rate of peripheral lipolysis coupled with a lack of an increased fat oxidation.[9,10] The extent to which triglycerides are produced and excreted into the blood in very low density lipoproteins (VLDLs) also is important in determining net fat accumulation in the liver. Net muscle protein synthesis and wound healing are promoted in burned children by the administration of exogenous recombinant human growth hormone (rhGH).[11–13] The beneficial effects of rhGH treatment, however, could be theoretically offset to some extent by the fact that rhGH has been shown to stimulate lipolysis,[14–17] which could lead to even greater deposition of fat in the liver.

The increase in basal lipolysis in burn patients is caused by excessive catecholamine production, which can be diminished by beta-adrenergic blockade with propranolol.[9,10,18] Propranolol administered to burn children in doses that effectively decrease peripheral lipolysis also has been shown to have salutary cardiovascular effects.[19] The mechanisms by which growth hormone stimulates lipolysis is not clear; thus, it currently is not known whether propranolol can inhibit lipolysis in patients receiving rhGH. Furthermore, all previous studies of lipolysis in burn patients have been in the fasting state, yet most patients are fed continuously, and given the insulin response to food intake, lipolysis could potentially be inhibited.[20] Thus, the interaction of growth hormone and propranolol in the fed state may not be well predicted from the results of fasting studies. If propranolol inhibits lipolysis and thereby limits hepatic fatty acid uptake, it will only exert a beneficial effect on the hepatic accumulation of triglycerides (TGs) if the VLDL-TG output is maintained at a rate greater than the fatty acid uptake by the liver. This study investigates the effect of propranolol on lipolysis in burned children receiving rhGH as part of their clinical treatment and relates the suppressive effect of propranolol on lipolysis to the rate of secretion of VLDL-TG from the liver.

METHODS

Patients

Six burn patients admitted to the Shriners Burns Institute in Galveston, Texas, were studied during their acute postburn hypermetabolic phase. The patients (four males and two females) received rhGH treatment throughout the study and propranolol for 6 days in a crossover randomized trial. Another six burn patients of similar age, weight, and sex distribution served as controls, receiving similar clinical treatment, but not propranolol or rhGH. The mean burn size was 64% ± 7% total body surface area (TBSA) for the control group and 53% ± 8% TBSA for the treatment group. Burn surface area in the control and treatment groups was 51% third-degree burn. Patient's characteristics in the treatment group are summarized in Table 1. All patients were hemodynamically stable, and no patients had significant systemic infection, as confirmed by negative results from standard bacteriologic screening tests, normal neutrophil counts, and the absence of clinical signs of infection before and after the study period. All controls and patients receiving therapy survived.

This study was approved by the Institutional Review Board of the University of Texas Medical Branch and the Shriners Hospitals for Crippled Children. The parents were informed of the nature, purpose, and possible risks involved in the study, and informed consents were obtained before the patients entered the study.

Experimental Design

An 8-hour isotopic tracer infusion was performed during the sixth day of treatment with rhGH or during the sixth day of treatment with rhGH and propranolol. To avoid fluctuations in other parameters that may have affected metabolism in the clinical course of the patients, the sequence of treatment periods was randomized. For the same reason, each treatment period was started the day after an excision and grafting procedure. The infusion studies in both periods were performed on the sixth postoperative day (Fig. 1).

Recombinant human growth hormone ($0.2\ mg \times kg^{-1} \times day^{-1}$) was administered subcutaneously once a day throughout the study period in the treatment group. Propranolol was administered intravenously at 8-hour intervals for 6 days at an initial dose of $2\ mg \times kg^{-1} \times day^{-1}$ and subsequently titrated to achieve a 20% reduction in baseline heart rate. Thus, each patient was studied twice during the treatment period.

Patients in the treatment and control groups were studied during continuous enteral feeding. Throughout the study period, the patients were fed a carbohydrate-rich enteral diet (Vivonex T.E.N., Sandoz Nutrition Corp., Minneapolis, MN) through nasogastric or duodenal catheters, providing 1.4 times as many calories as the measured resting energy expenditure. The composition of the enteral diet was 15% of calories as free amino acids, 82% of calories as carbohydrate from partially hydrolyzed corn starch, and 2% of calories as fat (2.0%

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Table 1. PATIENT CHARACTERISTICS

	Age	Weight	Sex	Burn Size (% TBSA)	Burn Size (% 3°)
Control (saline)	10	48	M	42	42
	7	23	F	51	43
	5	25	M	60	43
	15	42	M	80	49
	17	50	F	85	60
	5	18	M	70	70
Treated (GH/propranolol)	10	40	M	34	31
	7	20	F	37	34
	8	33	M	35	35
	10	35	M	54	54
	5	20	M	75	71
	13	71	F	80	80

TBSA = total body surface area, GH = growth hormone.

linoleic acid). The concentration of Vivonex (kcal/mL) was individually adjusted so that there were no clinical symptoms, such as diarrhea. A comparable high-carbohydrate/low-fat diet previously has been shown to eliminate the influx of exogenous fat via absorption,[21] thus the fatty acids as well as the TGs that appear in plasma under this dietary condition are from endogenous sources.

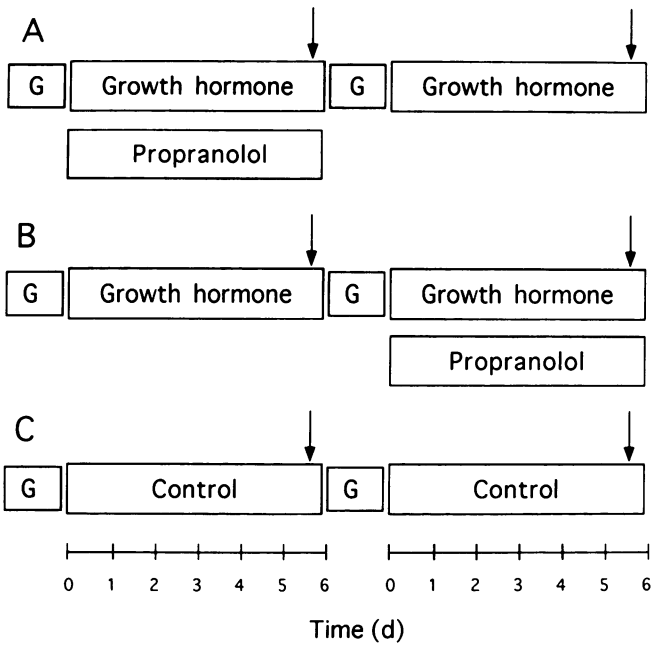

Figure 1. Schematic representation of the study design. To avoid the fluctuation of other parameters due to differences in clinical course of the patients, the six patients of the treatment group were randomized into two treatment modalities (A),(B). A comparable group of six patients served as controls (C)—*i.e.*, no recombinant human growth hormone or propranolol treatment. To control for operative treatment (excision and grafting) all isotope infusion studies were performed 6 days postoperatively. G = grafting; downward arrow = infusion study.

The experimental protocol for each 8-hour isotopic tracer infusion is illustrated schematically in Figure 2. The patients received a prime of (60 μmol $\times$ kg^{-1}) followed by an 8-hour continuous (2 μmol $\times$ kg^{-1} $\times$ minute^{-1}) infusion of [1,2-^{13}C] acetate (99% enriched; Isotec Inc., Miamisburg, OH), and a 1-hour continuous (0.04 mmol $\times$ kg^{-1} $\times$ minute^{-1}) infusion of [1-^{13}C]-palmitate (99% enriched; Cambridge Isotope Laboratories, Andover, MA) during the last hour of the infusion study. Blood samples were drawn from femoral artery catheters at 0, 60, 90, 120, 150, 180, 360, 390, 420, 450, 465, 470, 475, and 480 minutes after the start of the isotopic tracer infusion for the determination of the isotopic enrichment of free plasma palmitate and palmitate bound to VLDL TGs. Both isotopes were infused through a central venous catheter using calibrated syringe pumps (Harvard Apparatus, Natick, MA). Indocyanine green (Cardiogreen, Becton Fickenson Microbiology Systems, Cocksville, MD) dissolved in 0.9% saline containing 5% human albumin was infused continuously at a rate of

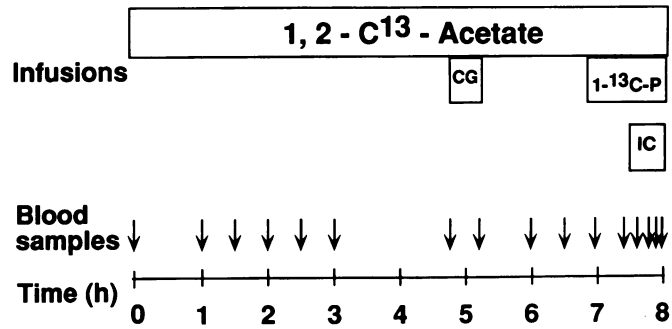

Figure 2. Schematic representation of the infusion study protocol. Uniformly labeled acetate and 1-13C-palmitate was infused continuously for lipid kinetics studies. CG = cardiogreen dye; 1-13C-P = 1-13C-palmitate; IC = indirect calorimetry.

0.25 mg × minute^{-1} for 20 minutes, starting at 360 minutes, to determine hepatic clearance of the dye. Blood samples were taken at 365, 370, 375, and 380 minutes from the central venous catheter. The concentrations of the dye in the infusate and in plasma samples were determined using a spectrophotometer set at 805 nm. Blood samples for epinephrine and norepinephrine concentrations were collected at the end of the infusion study, and the plasma was stored with 5-mg sodium metabisulfite. Indirect calorimetry was performed using a DeltaTrac Metabolic Cart (Sensormedix Corp., Yorba Linda, CA) during each infusion study. Measurements were made in the canopy mode for at least 10 minutes continuously between 420 and 480 minutes after the beginning of the tracer infusion.

Analysis of Samples

Very low density lipoproteins were isolated from 3 mL of plasma by overlaying the plasma with a density = 1.006 solution (0.9% sodium chloride) and spinning it in a 70.1-Ti rotor at 50,000 rpm (171 500 g average) for 20 hours at 15 C in an ultracentrifuge (Model L7-55 Beckman Ultracentrifuge, Beckman Instruments, Palo Alto, CA). After ultracentrifugation, the VLDL TG was carefully removed, along with the density solution found on top of the tube, by the slicing tube technique.[22] The total volume of the resulting VLDL suspension was 3 mL.

The TG concentrations in the VLDL suspension and in plasma were determined enzymatically (RA-500, Technicon Instruments Corp., Tarrytown, NY). Plasma glucose and lactate concentrations were measured using an automated glucose and lactate analyzer (Yellow Springs Instrument Inc., Yellow Springs, OH). Plasma insulin concentration was measured by radioimmunoassay (Instar Corp., Stillwater, MI). Epinephrine and norepinephrine were extracted from the plasma with alumina and separated by using Waters chromatography reverse-phase column. The samples were quantified using a model 460 electrochemical detector. Values were corrected for recovery using 3,4-dihydroxybenzylamine as an internal standard. Plasma β-hydroxybutyrate concentration was determined enzymatically (Sigma Diagnostics, St. Louis, MO).

Free fatty acids (FFAs) were extracted from plasma, isolated by thin-layer chromatography, and derivatized to fatty acid methyl esters. Palmitate and total FFA concentrations in plasma were determined by gas chromatography (Model 5890, Hewlett-Packard Co., Palo Alto, CA) using heptadecanoic acid as an internal standard. Triglycerides in the VLDL suspension were isolated by thin-layer chromatography, hydrolyzed to FFAs, and derivatized to fatty acid methyl esters. Relative concentrations of individual fatty acids in the VLDL fraction were determined by gas chromatography (Model 5890, Hewlett-Packard Co., Palo Alto, CA). Isotopic enrichment of plasma palmitate and palmitate bound to VLDL TGs was determined by gas chromatography-mass spectrometry (Model 5992, Hewlett-Packard Co., Palo Alto, CA) in the electron impact ionization mode for the ultimate computation of the tracer/tracee ratio. For the palmitate methyl ester, the ions of mass-to-charge ratio *(m/e) 270, 271,272, 273* and *274* were selectively monitored.

Calculations

Indirect calorimetry

Total energy expenditure and fat oxidation rates were calculated using stoichiometric equations.[23,24] Fat oxidation (f) is calculated using the formula:

$$f(g/min) = 1.67VO_2 - 1.67VCO_2 - 1.92n \quad (1)$$

and total energy expenditure was determined by using the formula:

$$TEE(cal/kg/min) = 3.9VCO_2/REE + 1.11VCO_2 - 2.17n \quad (2)$$

where VO_2 and VCO_2 are total oxygen consumption and CO_2 production in L/minute and respiratory exchange ratio (RER) is the ratio of VCO_2 to VO_2. Urinary nitrogen excretion (n) is equal to mg/minute. During each infusion study, the arteriovenous nitrogen balance across the leg was measured and, in both study groups, found to be close to zero (D. Chinkes, A. Aarsland, R. Wolfe, D. N. Herndon, unpublished data, 1996). Therefore, nitrogen excretion rate was assumed to equal nitrogen intake during the study period. This observation supports the notion that the patients maintained a state of protein balance—*i.e.*, nitrogen excretion rate was equal to nitrogen intake. A 30% error in the assumed nitrogen excretion rate value would have no effect on the calculated values of fat synthesis/oxidation or carbohydrate oxidation during this study.

It has been shown that when indirect calorimetry is used during net fat synthesis (the ratio of VCO_2 to VO_2, excluding their components from protein oxidation is >1; *i.e.*, with nonprotein respiratory exchange ratios [RERs] >1), the apparent rate of fat oxidation, (f), is the difference between the rate of fat oxidation (fo) and fat synthesis (fs) (f = fo − fs). With nonprotein respiratory exchange ratio > 1, the equation for fat oxidation yields a negative number. Thus, calculated rates of negative fat oxidation quantitatively represent net rates of fat synthesis.[23,24]

Hepatic Clearance of Indocyanine Green

Hepatic clearance of indocyanine green was calculated as rate of infusion divided by plasma concentration.[25]

Calculation of Lipid Kinetics

Sample analysis indicated that a physiologic and isotopic steady state existed during the infusion period in all studies, indicating that neither the concentration nor the enrichment changed during the time in which the samples were obtained. The rate of appearance of palmitate and the rate of secretion VLDL-bound palmitate were calculated with use of steady state equations.[26] The total rate of release of fatty acid (Ra FFA) into the systemic circulation was calculated by dividing the rate of appearance of palmitate (Ra palmitate) by the ratio of the concentrations of palmitate to total FFAs. This measurement of the rate of release of fatty acids is not necessarily the same as the rate of lipolysis, because some fatty acids can be re-esterfied within the adipose tissue.

Very low density lipoprotein TGs secreted by the liver are principally derived from two sources of fatty acid: 1) fatty acids that are synthesized *de novo* in the liver and 2) fatty acids not synthesized in the liver but derived from other sources such as diet and adipose tissue. In our experiment, fat was omitted from the diet so that the plasma FFA was entirely derived from lipolysis. Our method of measuring the kinetics of VLDL-bound TGs takes advantage of the fact that acetyl-CoA is the precursor for *de novo* fatty acid synthesis. Labeled acetate, mixed with the endogenous pool of acetyl-CoA, labels newly formed fatty acids in the liver. A certain fraction of these labeled fatty acids are secreted from the liver as VLDL-bound fatty acids. If the enrichment of the precursor pool is known, then the rate of secretion of *de novo* synthesized fatty acids can be calculated by measuring rate of incorporation of the label into the product. The enrichment of the hepatic precursor pool for fatty acid synthesis cannot be measured directly or even estimated *in vivo*. The novelty of the current method is that the precursor enrichment is based on the "enrichment pattern" (isotopomer distribution) of the product (VLDL-bound fatty acids) as outlined in this section. This enables us to measure the rate of secretion of the individual *de novo* synthesized fatty acids and the total rate of secretion of the individual VLDL-bound fatty acids based on the dilution principle. The rate of secretion of re-esterified fatty acids is calculated by subtracting the rate of secretion of *de novo* synthesized fatty acids from the total rate of secretion of the individual fatty acids. In the current study, kinetics for palmitate are reported because palmitate is the predominate product of *de novo* fat synthesis and also serves as a representative fatty acid for the re-esterification pathway. The total rate of VLDL-TG secretion is obtained by dividing the total rate of palmitate secretion by the percentage of VLDL-bound palmitate.

Rate of Secretion of *De Novo* Synthesized Palmitate Bound to Very Low Density Lipoprotein Triglyceride

The precursor enrichment is deducted essentially as described by Hellerstein et al.[27,28] Briefly, we infused [1,2-^{13}C] acetate for 8 hours and measured the M+0, M+2, and M+4 enrichments (the palmitate with +0, +2, and +4 mass units) of VLDL-bound palmitate over time. We chose to use doubly labeled acetate, as opposed to [1-^{13}C] acetate as used by Hellerstein et al.[27,28] because the precision of analysis was greatly improved because of minimal contribution of enrichment at the M+2 to the M+4 peak. Also, the background abundance of these isotopomers is very low.

The general principle behind the calculation of the precursor enrichment relies on the fact that eight acetyl-CoA molecules are necessary to form one palmitate molecule. Thus, if labeled acetate is infused, palmitate will be produced that has one, two, three, or more labeled biocarbons from acetate molecules. The relative abundance of palmitate labeled with one, as opposed to two acetate molecules will depend on the precursor enrichment, so that one can work backward and deduce the precursor enrichment from the relative abundance of palmitate labeled with one or two labeled acetate molecules. For example, if most of the palmitate molecules in a sample contain four labeled acetate molecules, one would conclude that 50% of the acetate is labeled. The exact formula we used to determine precursor enrichment (p) is:

$$p = [2 \times TTR(M + 4)/TTR(M + 2)]/$$
$$[(7 + 2 \times TTR(M + 4))/TTR(M + 2)] \quad (3)$$

where TTR(M + 4) and TTR(M + 2) are the tracer/tracee ratios of VLDL-bound palmitate that contain one or two labeled acetate molecules, respectively.

To calculate the fractional synthesis rate (FSR) of VLDL-bound palmitate, we used the formula:

$$FSR = [(E_{(t_2)} - E_{(t_1)})/(t_2 - t_1)]/[8p(1 - p)^7] \quad (4)$$

where t_1 and t_2 are the times when samples are taken and $E_{(t)}$ is the singly labeled enrichment at time t.

The differences between the formula that we used and the traditional formula[29] for calculation of FSR are the factor of 8 in the denominator and the factor $(1 - p)$.[7] The factor of 8 accounts for the fact that it requires eight acetate molecules to form one palmitate molecule. The factor of $(1 - p)^7$ accounts for the probability that seven acetate molecules that are not labeled will be incorporated into a palmitate molecule. To obtain absolute synthesis rates, the fractional synthetic rate was multiplied by the pool size of VLDL-bound palmitate. The pool size was estimated by multiplying the measured value of VLDL-TG concentration by the relative concentration of palmitate in the VLDL-TG fraction times the assumed plasma volume of 37 mL/kg.[30]

Table 2. MEAN VALUES ± STANDARD ERROR OF THE MEAN FOR TEMPERATURE, HEART RATE AND BLOOD PRESSURE

	Control	GH	GH + Propranolol
Temperature (°C)	38.6 ± 0.8	39.2 ± 0.4	39.0 ± 0.4
Heart rate ($B \cdot min^{-1}$)	169 ± 6	152 ± 8	119 ± 8*
Blood pressure (mmHg)			
Systole	129 ± 9	132 ± 11	116 ± 10
Diastole	70 ± 6	79 ± 3	65 ± 8

GH = growth hormone.
* Significant difference compared to GH and control at p = 0.001, n = 6 in each group.

Total Rate of Very Low Density Lipoprotein-Bound Palmitate Secretion

The total rate of VLDL-bound palmitate secretion rate is calculated from the measured rate at which *de novo* synthesized palmitate is secreted into the VLDL palmitate pool. Briefly, we can obtain the percentage of VLDL-bound palmitate, which is derived from *de novo* produced palmitate (% de novo VLDL palmitate) from the extent of dilution that occurs between the precursor and the product. The specific formula is:

$$\%\ \text{d.n. VLDL palmitate} = \text{plateau enrichment of m} + 2\ \text{labeled palmitate}/(8 \times \text{p}) \quad (5)$$

The total rate of VLDL-bound palmitate secretion is then calculated by dividing the rate at which *de novo* synthesized palmitate is secreted as VLDL by the percentage of VLDL-bound palmitate, which is derived from *de novo* produced palmitate (% d.n.VLDL palmitate). If a plateau is not reached during the infusion time, the extrapolated value for the plateau enrichment was used to calculate the total secretion rate.

Statistical Analysis

For statistical evaluation, Tukey's multiple comparisons, least squares linear regression analysis, and Student's two tailed t test were used when appropriate. P values less than 0.05 were considered significant. All values in the text, tables, and figures are expressed as means ± standard error of the mean.

RESULTS

In a crossover study, six burned children received 0.2 mg/kg/day rhGH and 0.2 mg/kg/day rhGH plus propranolol titrated to reduce the heart rate by 20% to 25% for 6 days, and six patients received placebo. Treatment and placebo groups were comparable for age, weight, burn size, and gender distribution (Table 1).

No differences in body temperature or blood pressure could be shown between treatments. Heart rate was titrated down with propranolol from a mean of 152 ± 8 beats/minute to 119 ± 8 beats/minute. This was an average reduction of 22% (Table 2). Figure 3 shows the Ra FFA in burned children receiving saline (control), rhGH, or rhGH plus propranolol. Propranolol was shown to significantly decrease Ra FFA in burn patients receiving rhGH; 5.23± 3.17 μmol/kg per minute for rhGH and 2.24± 0.33 μmol/kg per minute for rhGH plus propranolol. Furthermore, the Ra FFA for rhGH plus propranolol was significantly lower than that for control at 3.56 ± 1.20 μmol/kg per minute.

In light of the decrease in Ra FFA, the mean concentration of FFA changed accordingly from 0.15± 0.11 mmol/L for rhGH to 0.059± 0.02 mmol/L for rhGH plus propranolol; this change was not significant (p = 0.07). In those receiving rhGH, propranolol could not be shown to affect β-hydroxybutytrate, glucose, or lactate plasma levels. These values were 0.04 ± 0.03 mmol/L *vs.* 0.05 ± 0.03 mmol/L for ketone body, 7.2 ± 0.9 mmol/L *vs.* 8.2 ± 0.9 mmol/L for glucose, and 2.4 ± 0.4 mmol/L *vs.* 2.5 ± 0.9 mmol/L for lactate in the rhGH and rhGH plus propranolol treatment groups, respectively. The ketone body concentrations (β-hydroxybutytrate) are of particular importance because they serve as indicators for the rate of fat oxidation in the liver. Plasma glucose and lactate, which are alternative energy sources for the liver, were unchanged with propranolol. Although total plasma TGs were in the upper normal range, they were not affected with propranolol treatment: 1.39 ± 0.26 mmol/L with rhGH and 1.76 ± 0.29 with rhGH + pro-

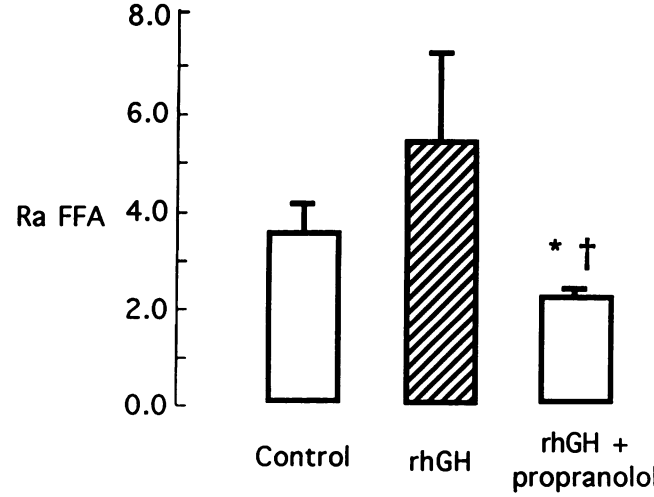

Figure 3. Rate of release of free fatty acids ([Ra FFA], μmol/kg/min). *Significantly different at p < 0.05 compared with recombinant human growth hormone (rhGH). *Significantly different at p < 0.05 compared with control. Values are means ± standard error of the mean (n = 6).

Table 3. MEAN VALUES ± STANDARD ERROR OF THE MEAN FOR RESTING ENERGY EXPENDITURE, RESPIRATORY EXCHANGE RATIO, CARBOHYDRATE OXIDATION AND WHOLE BODY NET FAT SYNTHESIS

	GH	GH + Propranolol
REE (cal/kg/min)	33 ± 11	28 ± 8
RER	0.98 ± 0.04	1.01 ± 0.03
Carbohydrate oxidation (mg/kg/min)	6.9 ± 1.1	7.1 ± 1.0
Whole body net fat synthesis (mg/kg/min)	0.2 ± 0.3	0.5 ± 0.2

GH = growth hormone; REE = resting energy expenditure; RER = respiratory exchange ratio.

pranolol. Neither were VLDL TGs changed: 0.49 ± 0.13 mmol/L for rhGH compared with 0.72 ± 0.51 for rhGH + propranolol; thus, the relationship of VLDL-TG to the total plasma TG remained the same for rhGH and rhGH plus propranolol treatments. Indirect calorimetry was done during ongoing feeding with a high carbohydrate diet. The calculated values for respiratory exchange ratio, resting energy expended, carbohydrate oxidation, and fat oxidation/synthesis were not influenced by propranolol treatment (Table 3). The respiratory exchange ratio of approximately 1.0 in both treatment groups indicates that carbohydrate was the main energy substrate. The low rate of net fat synthesis at the whole-body level further indicates that the patients were in energy balance.

Palmitate is the predominant product of fatty acid synthesis. The rate of synthesis and subsequent secretion of palmitate in the form of VLDL-TG was not significantly affected with propranolol (Table 4); neither was the rate of secretion of palmitate, through the re-esterification pathway, from the plasma free fatty acid pool. Thus, total rate of secretion of VLDL-TG-bound palmitate was not changed by propranolol. In both treatment groups, secretion of newly formed palmitate did not account for more than approximately 20% of the total secretion of palmitate. Similar to the secretion of palmitate, the secretion rate of total VLDL-TG was not affected by propranolol treatment.

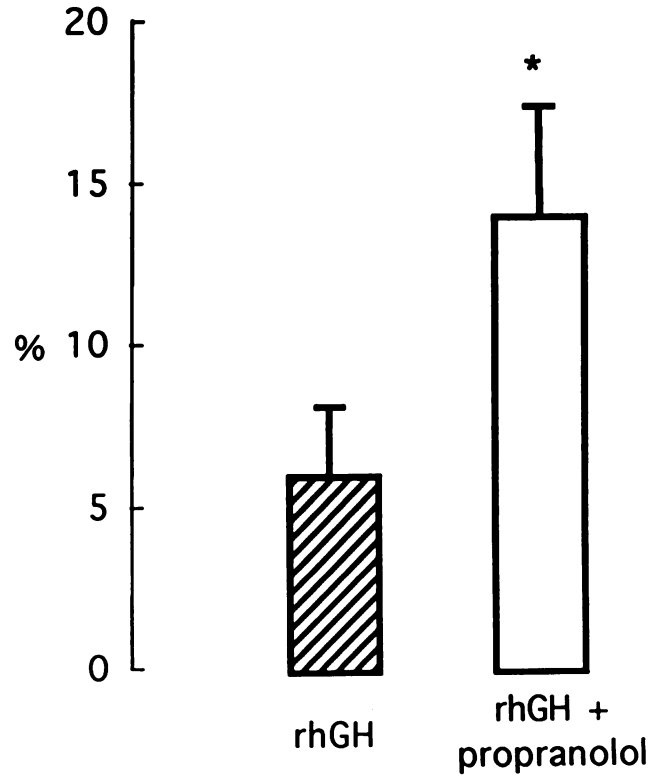

Figure 4. Percent of total peripheral palmitate release that is re-esterified by the liver into very low density lipoprotein triglycerides. *Significantly different at $p < 0.05$. Values are means ± standard error of the mean ($n = 6$). rhGH = recombinant human growth hormone.

Figure 4 depicts the percentage of total peripheral palmitate release that is re-esterified by the liver into VLDL-TG. Although propranolol decreased the rate of release of palmitate by nearly 60% (Table 4), hepatic secretion of re-esterified fatty acids remained the same. This indicates that the liver was nearly 60% more effective in secreting re-esterified fatty acids relative to Ra FFA.

Table 4. MEAN VALUES ± STANDARD ERROR OF THE MEAN FOR LIPID KINETICS

	GH	GH + Propranolol
Rate of appearance of palmitate (μmol FA/kg/min)	1.73 ± 1.24	0.74 ± 0.11*
Secretion of de novo synth. palmitate (μmol FA/kg/day)	35 ± 11	25 ± 11
Secretion of reesterified palmitate (μmol FA/kg/day)	138 ± 51	153 ± 38
Total secretion of palmitate (μmol FA/kg/day)	173 ± 57	177 ± 38
Total secretion of VLDL-TG (μmol TG/kg/day)	192 ± 65	213 ± 40
Rate of plasma clearance of VLDL-TG (L/kg/day)	0.39 ± 0.06	0.34 ± 0.06

GH = growth hormone; VLDL-TG = very low density lipoprotein-triglycerides.
* Significant difference at $p < 0.05$, $n = 6$ in each group.

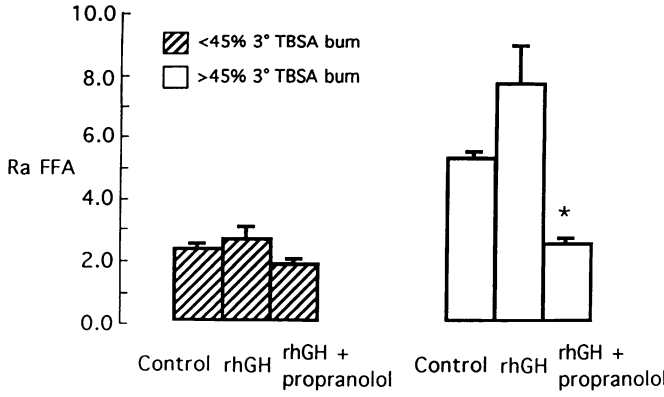

Figure 5. Rate of release of free fatty acids (Ra FFA, μmol/kg/min). *Significant difference at $p < 0.05$ compared with recombinant human growth hormone (rhGH). Values are means ± standard error of the mean (n = 3). TBSA = total body surface area.

The relationship between the Ra FFA and third-degree burn size was analyzed by least squares linear regression analysis, which showed that in the larger burns, Ra FFA was larger compared with the smaller burns. When experimental measurements for Ra FFA and catecholamines in the less than 45% TBSA were compared with burns greater than 45% TBSA, the pharmacodynamic effects were more pronounced. Figure 5 depicts the Ra FFA for third-degree burns covering less than 45% TBSA and for those that are greater than 45% TBSA. Control values were 2.73 ± 0.11 μmol/kg per minute and 5.25 ± 0.06 μmol/kg per minute, respectively. The Ra FFA for smaller and larger burns receiving rhGH was 2.80 ± 0.44 μmol/kg per minute and 7.67 ± 1.08 μmol/kg per minute, respectively; for those patients receiving rhGH plus propranolol, values were 1.95 ± 0.08 μmol/kg per minute and 2.53 ± 0.04 μmol/kg per minute, respectively.

The plasma FFA concentrations depicted in Figure 6 were significantly different at $p < 0.05$ in the larger burn group for controls and growth hormone but not for those

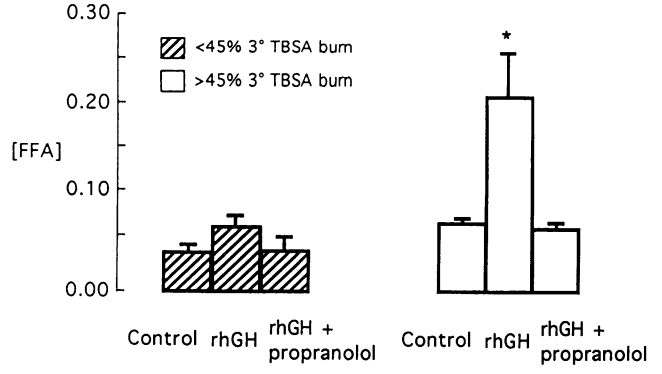

Figure 6. Plasma free fatty acid ([FFA]) concentrations (mmol/L). *Significantly differently at $p < 0.05$ compared with control and recombinant human growth hormone (rhGH) + propranolol. Values are means ± standard error of the mean (n = 3). TBSA = total body surface area.

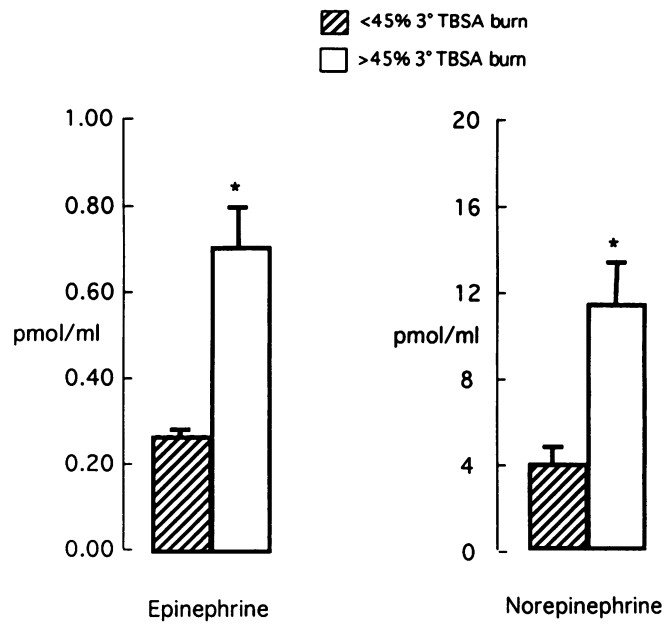

Figure 7. Epinephrine and norepinephrine concentrations with recombinant human growth hormone. *Significantly different at $p < 0.05$. Values are means ± standard error of the mean (n = 3). TBSA = total body surface area.

receiving rhGH plus propranolol. Values for FFA concentrations for the smaller burns were 0.0450 ± 0.0012 mmol/l, 0.071 ± 0.007 mmol/L, and 0.049 ± 0.010 mmol/L for controls, rhGH, and rhGH plus propranolol, respectively; for the larger burns, the values were 0.076 ± 0.0006 mmol/L, 0.227 ± 0.046 mmol/L, and 0.068 ± 0.001 mmol/L for controls, rhGH, and rhGH plus propranolol, respectively.

When patients receiving rhGH with third-degree burns over 45% TBSA were compared with those with third-degree burns less than 45% TBSA (Fig. 7), epinephrine and norepinephrine concentrations were significantly higher ($p < 0.05$, at 0.72 ± 0.13 pmol/mL *vs.* 0.29 ± 0.09 pmol/mL, and 11.5 pmol/mL *vs.* 3.87 ± 0.63 pmol/mL, respectively). No significant difference in epinephrine and norepinephrine concentrations could be demonstrated when the patients were treated with rhGH plus propranolol between large burns (0.50 ± 0.27 pmol/mL *vs.* 11.30 ± 5.70 pmol/mL respectively) and small burns (0.40 ± 0.09 pmol/mL *vs.* 3.54 ± 0.21 pmol/mL, respectively).

DISCUSSION

Fatty liver occurs in severely burned patients, often resulting in twofold increases in liver size,[3,8] and this may cause clinical problems. Metabolic failure of the liver can complicate recovery from a burn injury or trauma, particularly when steatosis develops because of an imbalance between the rate of TG formation in the liver and

the rate of secretion of VLDL-TG into the blood. The results of our study show that in severely burned children, plasma FFA is the primary precursor for hepatic TG synthesis. Although the patients received prolonged high-carbohydrate feeding, *de novo* synthesis of fatty acids played a minor role in the production of VLDL-TG. Free fatty acid delivery to the liver was augmented by the administration of rhGH, which was therapeutically given to the patients to promote wound healing.[12] Prolonged treatment of burn patients receiving rhGH plus propranolol effectively decreased the availability of FFA to the liver by reducing the release of fatty acids into the blood. Concomitantly, plasma FFA was converted more efficiently into VLDL-TG and secreted when propranolol was given along with rhGH. Principally, the net balance of fat across the liver is a function of FFA uptake from plasma, intrahepatic FA consumption (oxidation), and VLDL-TG secretion. Because hepatic FA uptake is closely correlated to plasma FFA availability, we propose that propranolol may have the beneficial effect of reducing net fat accumulation in the liver by decreasing hepatic FFA uptake while maintaining VLDL-TG secretion without affecting fat oxidation.

We previously documented that lipolysis is elevated in burned children in the postabsorptive state.[9,10] The results of the current study show that a high-carbohydrate enteral diet effectively suppresses lipolysis in children with burns less than 45% TBSA because the Ra FFA in those patients (2.8 ± 0.6 μmol $\times$ kg^{-1} $\times$ min^{-1}) was as low as would have been expected in healthy individuals in the same nutritional state.[31] The patients treated with rhGH who had burns greater than 45% TBSA experienced significantly higher values for Ra FFA. The principal parameter of interest with regard to delivery of FFA to the liver is plasma concentration, which was approximately fourfold greater in the patients with burns greater than 45% TBSA and treated with rhGH as compared with the controls or with patients with burns less than 45% TBSA who were not given rhGH. Because there was virtually no fat oxidation in any of the patients (due to high carbohydrate feeding), all of the fatty acids released were eventually re-esterified. Consequently, it is reasonable to expect that any increase in Ra FFA would be reflected directly in increased hepatic uptake and re-esterification into TG. The ability of propranolol to decrease Ra FFA in the patients with large burns who were given rhGH, therefore, most likely also reflects a marked decrease in hepatic fatty acid uptake.

The re-esterification of fatty acids released into the blood has previously been called the TG-fatty acid cycle.[32,33] We have shown that this cycle is elevated in burn patients in the fasted state.[9,33] The persistence of this cycle during high-carbohydrate feeding is striking because essentially there is no net forward direction of this cycle—*i.e.*, fat oxidation. It generally has been presumed that all re-esterification in the TG-FFA cycle occurs in the liver; however, our results indicate that, at maximum, no more than 15% of recycled fatty acids appeared in newly secreted VLDL-TG. This means that either a large portion of the fatty acids taken up in the liver remained there, or that fatty acid uptake and re-esterification also were occurring peripherally. To assess the extent to which the fat might be retained in the liver, we reviewed the autopsy reports of 14 severely burned children, ranging in age from 2 months to 15 years, who died 15 to 30 days after injury. There were 3 girls and 11 boys with 2 scald and 12 flame burns; 3 had concomitant smoke inhalation injuries. The mean burn size was 76% $\pm$ 5% TBSA (standard error of the mean). Liver weights were 75.6 ± 6.0 g/kg body weight in burn victims compared with 34.3 ± 1.1 for predicted healthy controls. We also examined, in a blinded study, histologic slides of the liver of the patients. Findings showed seven with diffuse, three with centrilobular, and two with periportal mild to marked liver fat changes. Mild to moderate cholestasis was shown in 5 of the 14 autopsies reviewed. We concluded that, in a severely burned child, the liver weight increases about 1 to 2 g $\times$ kg^{-1} $\times$ day^{-1}. The rate of release of FFA in the current study was found to be 3 g $\times$ kg^{-1} $\times$ day^{-1}, and the rate of secretion of VLDL-TG indicates that approximately 0.1 g $\times$ kg^{-1} of FFA could be accounted for by this route. Consequently, it appears the accumulation of fat in the liver occurs at a rate consistent with the measured imbalance between Ra FFA and the rate of secretion of VLDL-TG. It also is likely that some peripheral re-esterification occurs.

The data presented in this manuscript represent the first study in which TG kinetics have been quantified in burned or critically ill patients. We have used a method that not only allows quantitation of the rate of release of VLDL-TG, but also allows the distinction between the secretion of TG containing *de novo* synthesized fatty acids, as opposed to re-esterified plasma FFA. We found that even during a high-carbohydrate diet, the total rate of newly synthesized fatty acids appearing in VLDL-TG was less than 0.07 μmol/kg per minute. If we assume that this represents as little as 5% of total hepatic fatty acid synthesis, only 1.4 μmol FFA/kg per minute was produced by *de novo* synthesis in the liver. Thus, compared with the rate of release of FFA (8 μmol/kg/min), *de novo* synthesis of fatty acids is a minor component of total availability of fatty acids for liver TG synthesis. Only 20% of secreted fatty acids in VLDL-TG come from *de novo* synthesis. Consequently, the suppression of lipolysis would be expected to be the major mechanism by which reduction of fatty acids in the liver could be achieved.

Our method of quantifying VLDL-TG kinetics allowed us to calculate Ra FFA and to distinguish the relative contributions of *de novo* synthesis and lipolysis, but

the method also enabled us to calculate FFA-TG clearance. Triglyceride clearance is dependent on the enzyme lipoprotein lipase, which hydrolyzes the circulating TG so that fatty acids can be taken up at the tissue level. Lipoprotein lipase activity has been reported to be decreased in septic patients.[34] If this were true in burn patients, we would have seen a low rate of TG clearance. However, the VLDL-TG clearance in burn patients actually was elevated above the value we observed previously in healthy volunteers during a high-carbohydrate diet (A. Aarsland, D. Chinkes, R. R. Wolfe, unpublished observations, 1996). Consequently, we conclude that there is no functional deficiency in lipoprotein lipase activity in burn patients.

The measurement of rate of release of VLDL-TG also allowed us to determine if reducing Ra FFA by propranolol administration would be anticipated to reduce fat infiltration in the liver because fat accumulation is the balance between uptake and secretion. Although propranolol decreased fatty acid availability, rate of release of VLDL-TG was maintained, reflecting a significant increase in the efficiency with which plasma FFAs were cleared and secreted in VLDL-TG. Propranolol may have had a direct effect on the liver production of VLDL-TG. More likely, TG accumulation occurs in the livers of burn patients because of deficiency in the secretion of VLDL-TG. The rates observed in this study were considerably less than the maximal rate we have observed in healthy volunteers under similar nutritional conditions (A. Aarsland, D. Chinkes, R. R. Wolfe, unpublished observations, 1996). Furthermore, in the burn patients, the Ra FFA, and thus hepatic fatty acid availability, was several-fold less than that level we have observed in resting highly trained endurance athletes[35]; yet these athletes do not accumulate fat in the liver despite extremely high rates of re-esterification.[36] Thus, it seems likely that rate of release of VLDL-TG did not decrease when Ra FFA was reduced by propranolol because some other mechanism than FFA availability was limiting rate of release of VLDL-TG. In contrast, in healthy subjects, availability of plasma FFA is considered to be the primary determinant of rate of release VLDL-TG.[37–39].

The rationale for our use of a high-carbohydrate diet was that there is no deficiency in the ability of burn patients to oxidize glucose,[40] and we have established the anabolic effect of insulin on protein metabolism.[41] When both glucose and fatty acids are available, glucose is the preferred energy substrate.[42] Thus, in the current study, the burn patients given rhGH did not oxidize fat despite a persistent Ra FFA of approximately 6 $\mu mol \times kg^{-1} \times min^{-1}$. The inhibition by glucose of fat oxidation in the liver is indicated in these patients not only by whole body indirect calorimetry data, but also by the low ketone concentrations. Consequently, the fatty acids taken up by the liver were channeled into TG synthesis. Thus, the effect of the high-carbohydrate diet on TG production was less by way of the *de novo* synthesis of fatty acids than by virtue of channeling available fatty acids away from oxidation and toward synthesis. Substitution of fat for some of the carbohydrate in the diet likely would have caused a higher rate of fat oxidation, but this would not have been reflected in altered TG synthesis because the total delivery of fat to the liver would have been increased. We previously have shown that only approximately 10% of administered fat is oxidized directly in burn patients.[43] Thus, it is not surprising that the nature of the nonprotein calories in critically ill patients is not a determinant in hepatic fat accumulation.[3] Rather, the critical factors appear to be 1) persistent lipolysis and 2) a deficiency in VLDL-TG secretion. In that regard, we have shown that propranolol treatment can significantly decrease lipolysis, even when the patients are in the fed state receiving rhGH, and that propranolol increases the efficiency of the hepatic secretion of fatty acids VLDL-TG. The administration of propranolol is not only safe in burned children, but it also has salutary cardiovascular effects.[19] Consequently, we conclude that propranolol administration is beneficial for severely burned children receiving rhGH.

Acknowledgments

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Discussion

DR. BASIL A. PRUITT, JR. (San Antonio, Texas): President Thompson, Secretary Copeland, Fellows, and Guests. I would like to compliment Dr. Herndon and his colleagues on adding another chapter to their volume of clinical studies identifying how the metabolic response to injury can be modified to the benefit of the patient. They have pioneered the use of growth hormone as a means of preserving lean body mass and improving the healing of burn wounds and donor sites.

To evaluate the findings reported this morning and the authors' conclusions, we need additional information, so I have a few questions for the authors.

Other investigators have noted that in growth hormone deficient adults treated with growth hormone, there is an increase in lipid oxidation. Yet the authors this morning note no such effect, and I wonder if they attribute that to the hormonal milieu characterizing postinjury hypermetabolism.

In earlier studies, David, you have reported that recombinant human growth hormone was associated with an increase in circulating levels of insulin and catecholamines. In these patients, the addition of propranolol appeared to exaggerate those hormonal changes and was associated with a fourfold decrease in fat oxidation. That seems counterproductive. I wonder whether that altered the mixture of nutrients serving as energy sources and whether there were changes in the respiratory quotient (RQ) indicative of such. I also wonder whether another group of patients who are fed and given propranolol alone is needed to sort out the interactions between the two pharmacologic agents.

In that same line, I wonder whether this problem of steatosis