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Research Article

Dietary components responsible for the regulation of somatomedin-C in humans were assessed in five adult volunteers of normal weight who were fasted for 5 d on three occasions, then refed three diets of differing composition. The serum somatomedin-C decreased from a mean prefasting value of 1.85 +/- 0.39 U/ml (+/- 1 SD) to 0.67 +/- 0.16 U/ml at the end of fasting (P less than 0.005). After refeeding for 5 d with a normal diet, the mean serum somatomedin-C increased to 1.26 +/- 0.20 U/ml. A protein-deficient (32% of control), isocaloric diet resulted in a significantly smaller increase, to a mean value of 0.90 +/- 0.24 U/ml (P less than 0.05). A diet deficient in both protein and energy led to a further fall 0.31 +/- 0.06 U/ml. The changes in somatomedin-C during fasting and refeeding correlated significantly with mean daily nitrogen balance (r = 0.90). We conclude that both protein and energy intake are regulators of serum somatomedin-C concentrations in adult humans, and energy intake may be of greater importance. The correlation between changes in somatomedin-C and nitrogen balance suggests that the former are directly related to changes in protein synthesis and may be helpful in assessing the response to nutritional therapy.

Dietary Components that Regulate Serum Somatomedin-C Concentrations in Humans

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ABSTRACT Dietary components responsible for the regulation of somatomedin-C in humans were assessed in five adult volunteers of normal weight who were fasted for 5 d on three occasions, then refed three diets of differing composition. The serum somatomedin-C decreased from a mean prefasting value of 1.85±0.39 U/ml (±1 SD) to 0.67±0.16 U/ml at the end of fasting ($P < 0.005$). After refeeding for 5 d with a normal diet, the mean serum somatomedin-C increased to 1.26±0.20 U/ml. A protein-deficient (32% of control), isocaloric diet resulted in a significantly smaller increase, to a mean value of 0.90±0.24 U/ml ($P < 0.05$). A diet deficient in both protein and energy led to a further fall in mean serum somatomedin-C concentrations to 0.31±0.06 U/ml. The changes in somatomedin-C during fasting and refeeding correlated significantly with mean daily nitrogen balance ($r = 0.90$).

We conclude that both protein and energy intake are regulators of serum somatomedin-C concentrations in adult humans, and energy intake may be of greater importance. The correlation between changes in somatomedin-C and nitrogen balance suggests that the former are directly related to changes in protein synthesis and may be helpful in assessing the response to nutritional therapy.

INTRODUCTION

Nutritional status is one of the most important determinants of somatomedin concentrations in blood (1). Bioassayable somatomedin levels are low in children with protein-calorie malnutrition (2, 3) and in starved rats (4). Using a sensitive, specific radioimmunoassay (5), we have reported that obese human subjects fasted for 10 d have a marked decline in somatomedin-C (6), one member of the somatomedin family of peptides. In these subjects the fall in somatomedin-C correlated with the change in urea nitrogen excretion, suggesting that the concentration of immunoreactive somatomedin-C might be a useful indicator of nutritional status.

The dietary components involved in the modulation of serum somatomedin concentrations in humans have not been defined. Phillips et al. (7) have reported that refeeding starved rats with a balanced, energy-deficient diet limited the increase in bioassayable somatomedin, and that when energy is adequate, the rate of recovery of somatomedin is dependent on the distribution of nutrients in the diet. Using the specific somatomedin-C radioimmunoassay, Prewett et al. (8) observed that optimal intake of both protein and energy were needed to maintain somatomedin-C levels in young growing rats. The studies reported here were undertaken in humans to determine the relative importance of dietary intake of protein and energy in the regulation of somatomedin-C and to determine whether any relationship exists between somatomedin-C and nitrogen balance during refeeding.

METHODS

Five healthy volunteers, two men and three women who were within 15% of ideal body weight (9) participated in the study. The subjects, ages 23–34 yr, were studied in the Clinical Research Unit of the North Carolina Memorial Hospital, but were allowed to maintain their normal activities outside the hospital at times not required for feeding or blood collections. Subjects were asked to maintain their usual activity, but not to engage in strenuous exercise. After giving informed consent, each subject was fasted for 5 d, being permitted to take water ad lib. and 20 meq KCl daily. Following each 5-d fast, they were refed for 5 d with one of three different test diets. At the end of the 5-d refeeding period they were given a diet that approximated home intake for 15 d or more, before beginning another fast-refeed cycle.

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TABLE I
Composition of Refeeding Diets

	g protein/kg body wt	*kcal/kg body wt*	Total calories
Normal diet	1.35±0.07	35.3±0.6	1,800–2,610
Low protein, isocaloric to the normal diet	0.43±0.05	35.1±0.7	1,797–2,579
Low protein, calorie deficient	0.40±0.03	10.8±0.2	552–799

The refeeding diets were assigned in random order after each fast and were composed as shown in Table I. Dietary protein and energy content were determined from standard food value tables (10).

During fasting, body weight and urinary ketones were measured daily to insure adherence to the fasting regimen. During the fasting and refeeding periods, blood samples were drawn daily between 0700 and 0900 (before breakfast in the refeeding period). Samples were allowed to clot briefly at room temperature, promptly centrifuged, and aliquots of serum were submitted for measurement of potassium, bicarbonate, and blood urea nitrogen and stored at a −20°C until assayed for somatomedin-C, insulin, and growth hormone. Concentrations of immunoreactive somatomedin-C/insulinlike growth factor I (11) were determined as previously described (5, 12). Selected pools of sera from study subjects were chromatographed in 0.5 M acetic acid on a G-50 Sephadex column (1.6 × 74 cm; LKB Instruments, Inc., Rockville, MD) which had been equilibrated in 0.5 M acetic acid, pH 2.7. The fractions (4 ml each) eluting between a distribution coefficient (K_d) of 0 and 0.85 were neutralized with 0.2 ml of 10 N NaOH, and assayed for somatomedin-C. Small molecular weight somatomedin-C stripped from its binding proteins eluted between K_d 0.27 and 0.52, as described by Zapf et al. (13). Serum insulin and growth hormone concentrations were determined by radioimmunoassay using purified porcine insulin (815-D63-10; kindly provided by Dr. Mary Root, Eli Lilly & Co.) as standards and purified human growth hormone (HS 2019G; provided by the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, and the National Hormone and Pituitary Program of the University of Maryland). 24-h urine collections were obtained during the fasting and refeeding periods and were submitted for autoanalyzer determinations of creatinine and urea nitrogen (14, 15). Urinary ketones were determined by the nitroprusside reaction (16).

Nitrogen balance was estimated by the following formula: nitrogen balance = total nitrogen intake − (urinary urea nitrogen + 4 g) (17, 18). Student's *t* test was used in statistical analysis of data, with significance defined at the 95% level. Changes in nitrogen balance and somatomedin-C levels were correlated using linear regression analysis.

RESULTS

Fasting and refeeding were carried out without significant complications. One subject required the oral administration of 3–6 g of sodium bicarbonate daily during fasting to maintain serum bicarbonate > 13 meq/liter. Of the 15 fasts, 14 were accompanied by ketonuria by the morning of the 2nd d, and in the remaining fast the subject was ketonuric by the morning of the 3rd d. The mean weight loss during fasting was 4.3±1.3 kg (±1 SD). The mean serum somatomedin-C concentrations at the start and the end of fasting were 1.85±0.39 U/ml (±1 SD)[1] and 0.67±0.15 U/ml, respectively ($P < 0.005$; Fig. 1).

Refeeding with isocaloric diets (~2,300 calories) resulted in substantial increases in immunoreactive somatomedin-C. The increase, however, was greater when the subjects received the normal diet (0.56±0.07 U/ml; Fig. 1) than when they received the isocaloric, protein-deficient diet (0.26±0.04 U/ml; Fig. 1). The absolute difference between the two diets reached statistical significance by day 2 of refeeding ($P < 0.01$;

[1] Somatomedin-C levels vary during each decade of life and are relatively higher in the second and third decades than at any other time. The value of 1 U/ml of a standard serum pool is assigned on the basis of the somatomedin-C immunoreactivity of 220 normal adults, ages 18–64 yr. The mean value of our study subjects (1.85 U/ml) is <1 SD above the mean of 1.45 U/ml for adults ages 23–34 yr.

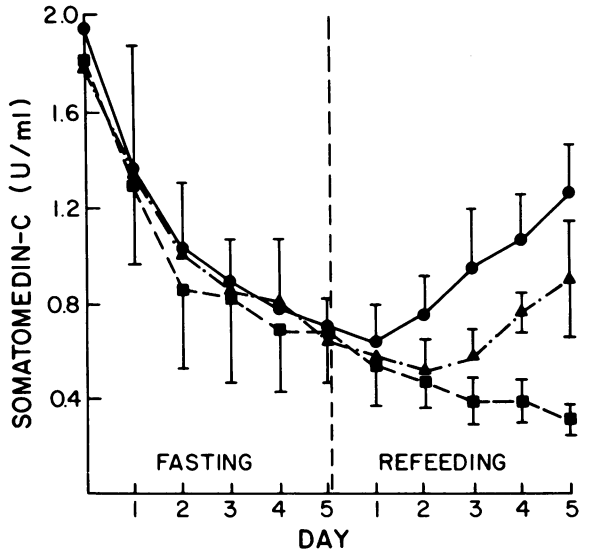

FIGURE 1 Serum somatomedin-C during periods of fasting and refeeding. Each point represents the mean±1 SD for the five study subjects. Normal diet (●); low protein, isocaloric diet (▲); low protein, low energy diet (■).

Fig. 1) and this difference remained relatively unchanged through the 5th d. Refeeding with the diet deficient in both protein and energy led to a further decline in somatomedin-C (difference of means for all patients, 0.37 U/ml). The mean difference in somatomedin-C concentrations between this diet and the normal energy, low protein diet was significant by day 3 of refeeding (0.18 U/ml, $P < 0.01$) and increased to 0.59 U/ml of day 5 (Fig. 1). The relative effects of refeeding protein and energy are depicted graphically in Fig. 2.

To rule out the possibility that the serum-binding proteins for somatomedin-C were distorting the results obtained in our radioimmunoassay (19), we prepared three pools of serum taken from all five subjects before fasting, at the end of 5 d of fasting, and after refeeding with the normal diet for 5 d. These serum pools were then chromatographed on Sephadex G-50 in 0.5 M acetic acid. The immunoreactive somatomedin-C content of the acid-chromatographed serum pools and nonacid chromatographed samples showed similar relationships. Specifically, the nadir values at the end of fasting were 36 and 40% of control in native and the acid-chromatographed samples, respectively. The samples at the end of refeeding were 70 and 91% of the control in the native and the acid-chromatographed samples, respectively. This indicates that the somatomedin-C measured in native serum provides a reasonable approximation of that measured after acid chromatography.

Although there were individual variations in the increments of somatomedin-C with refeeding (Fig. 3), responses in four of the five subjects reflect those of the group as a whole for both of the normal energy diets. In the remaining subject the somatomedin-C increments were similar for both diets. This may reflect the fact that this subject's caloric requirements were greater while she was ingesting the normal diet, because she was engaging in unauthorized vigorous physical activity.

The mean daily nitrogen loss for all subjects during fasting was 11.7±2.1 g/d. While refeeding a normal diet, the daily estimated nitrogen balance became positive in all subjects with a mean nitrogen retention of 2.0 g/d. Nitrogen balance remained negative on the low protein, isocaloric diet with a mean deficit of 2.9 g/d. On the diet deficient in both protein and energy the mean nitrogen deficit was 5.2 g/d. Since somatomedin stimulates protein synthesis in vitro, cumulative changes in somatomedin-C levels and nitrogen balance during refeeding were analyzed by linear regression. Significant correlations were found for the normal diet ($r = 0.67$, $P < 0.001$) (Fig. 4) and for the low protein, isocaloric diet ($r = 0.54$, $P < 0.005$). A significant cor-

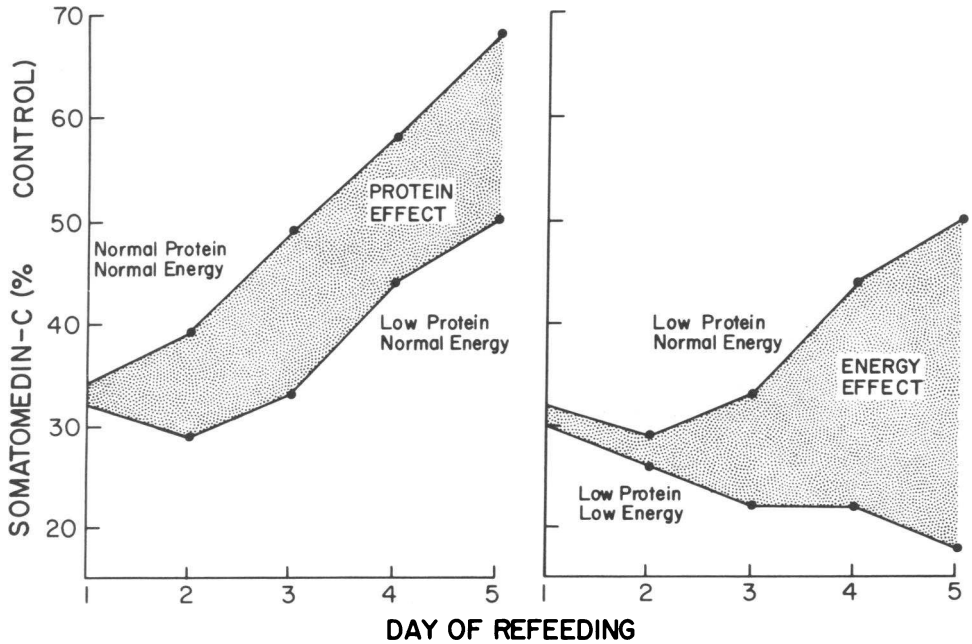

FIGURE 2 Comparison of the effects of protein (left panel) and of energy (right panel) on serum somatomedin-C during refeeding. Each point represents the percentage of control of the mean prefast value. The effect of protein is depicted as the difference between the two isocaloric diets. The effect of energy is the difference between the two protein-deficient diets.

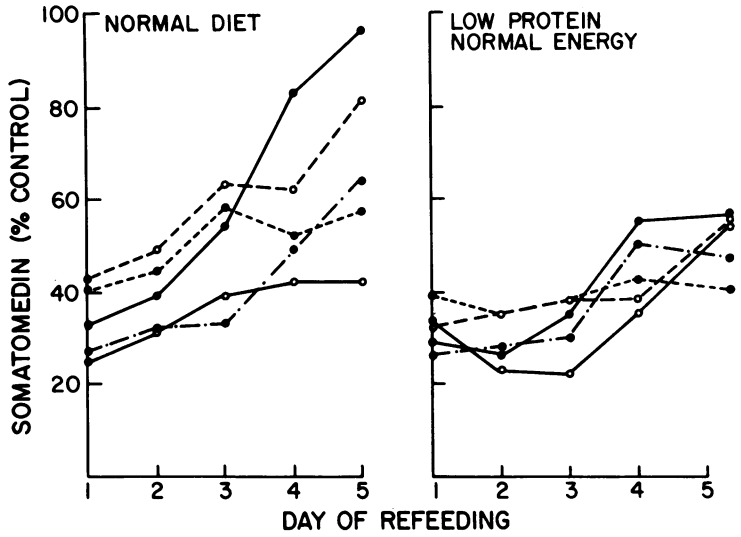

FIGURE 3 The response of each subject to refeeding the two normal energy diets. Somatomedin-C is expressed as the percentage of control of the mean prefast value. One subject (O) was engaged in vigorous physical activity while ingesting the normal diet.

relation was also found between changes in somatomedin-C during each fasting or refeeding period and mean daily nitrogen balance during that period ($r = 0.90$; $P < 0.001$) (Fig. 5). When assessed in this manner, it is apparent that it is possible for subjects to have mild negative nitrogen balance, but a positive increment in serum somatomedin-C. This occurs in subjects whose marked fasting-induced decline is followed by a rise in somatomedin-C while being refed the low protein-normal energy diet, a regimen on which ni-

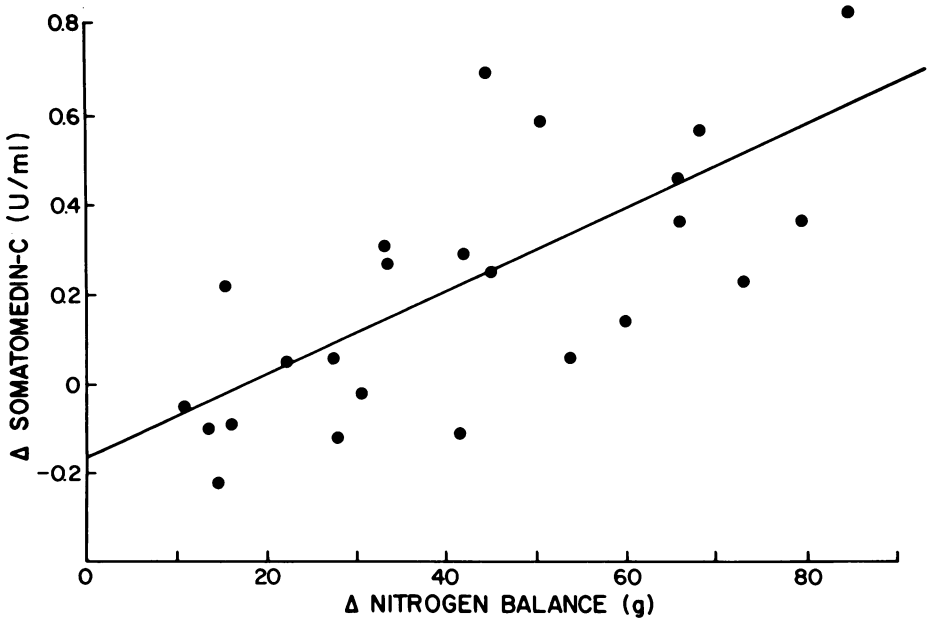

FIGURE 4 Correlation between the changes in somatomedin-C and nitrogen balance while refeeding the normal diet. The change in somatomedin-C is the difference for each subject between the value on the day of observation and the value at the end of fasting. The change in nitrogen balance is the sum of the differences between each day of observation and the mean daily loss during fasting. $r = 0.67$; $P < 0.001$.

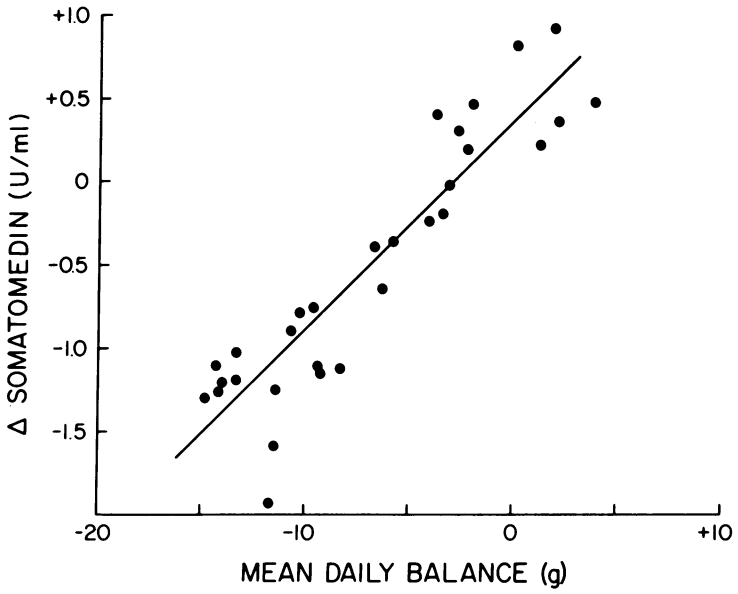

FIGURE 5 Correlation between somatomedin-C and nitrogen balance during all periods of fasting and refeeding. The change in somatomedin-C is the total 5-d change over either the fasting or refeeding periods. The mean daily nitrogen balance is the average daily nitrogen balance for each subject over each 5-d study period. $r = 0.90$; $P < 0.001$.

trogen balance remains negative. When the magnitude of change in somatomedin-C is compared with the change in nitrogen stores during fasting and refeeding of the normal diet, it is apparent that there are large shifts in somatomedin-C compared with changes in nitrogen stores (Table II). Somatomedin-C declines by 64% during fasting, while only 4% of total body nitrogen is lost. Refeeding a normal diet causes an 88% increase in somatomedin-C, while only 17% of the nitrogen lost during fasting is repleted.

To exclude the possibility that changes in serum somatomedin-C concentrations merely reflected changes in other hormones, both fasting insulin and growth hormone concentrations were determined for each day of the study period. During each of the three 5-d fasting periods, the mean basal insulin concentrations declined from control values and this change was significant during two periods (12.0±12.3 μU/ml to 2.2±1.7 μU/ml preceding the normal diet; and 11.3±13.3 to 3.2±2.0 μU/ml preceding the isocaloric, low protein diet). During refeeding, fasting insulin concentrations rose on all three diets by the end of the first refeeding day, and remained relatively constant thereafter. This increase was significant (from 2.2 up to 7.1 μU/ml; $P < 0.05$) only for the normal diet. When the changes in fasting insulin concentrations for each fasting and refeeding period were correlated with the mean daily nitrogen balance, the r value was 0.38 ($P < 0.05$).

The mean serum growth hormone concentrations showed a minimal increase (<3 ng/ml) during the first 3 d of each fasting period, then declined on the 4th and 5th d. With refeeding, two subjects had suppression of fasting growth hormone values and three others were unchanged. None of these changes reached statistical significance.

TABLE II
Relationship between the Change in Somatomedin-C and the Change in Total Body Nitrogen Stores

	Fasting	Refeeding
Somatomedin-C	64% decrease	88% increase
Total body nitrogen*	4% decrease	17% deficit restored

* Total body nitrogen estimates assume that the total body mass is 16.4% protein (20).

DISCUSSION

The decline in serum immunoreactive somatomedin-C observed in normal weight subjects during fasting confirms our previous results with obese subjects (6), but is more accelerated. Since this decline occurs when there has been little reduction in protein and fat stores, there is reason to believe that the serum concentration of this growth factor may be a sensitive indicator of acute changes in nutritional status. Refeeding a normal

diet for 5 d resulted in the return of somatomedin-C to 67% of prefast values. The rate of this rise in somatomedin-C was constant after the 1st d of refeeding. After a 2-d delay, the isocaloric diet, which was deficient in protein, also produced a comparable rate of increase in somatomedin-C. In the presence of adequate energy, therefore, optimal intake of protein appears to exert its greatest effect early in refeeding. The 2-d lag period may be the time required to reach a new steady state of improved protein absorption and/or utilization, a common adaptation to low protein-adequate energy diets (21). Return of somatomedin-C toward control levels following this period might reflect the body's more efficient utilization of a subnormal dietary protein intake.

An adequate supply of energy appears to be essential for the rise in somatomedin-C after fasting. This is suggested by the finding that, compared with the rise noted in subjects refed a low protein-adequate energy diet (35 kcal/kg), feeding the diet in which energy was restricted (10.8 kcal/kg) caused a further decline. In keeping with our findings in humans, Phillips and Vassiloupoulou-Sellin (22) have reported that refeeding adequate energy to starved rats is necessary to effect a sustained rise in somatomedin and that protein deficiency results in only a transient increment in somatomedin activity. Prewitt et al. (8) have shown that both protein and energy are important in maintaining immunoreactive somatomedin-C in rats 12 or more weeks old. In younger animals, protein appears to be more important than energy in this regard, a finding consistent with the age-dependent need for protein (23). Our findings along with those of others (7), therefore, suggest that both protein and energy are needed to restore somatomedin-C after fasting, and that a minimum quantity of energy appears to be necessary for this increase.

The strong correlations among cumulative changes in somatomedin-C and cumulative changes in nitrogen balance during refeeding, as well as the excellent correlation between change in somatomedin-C and mean daily nitrogen balance during fasting and refeeding, provide further evidence that changes in somatomedin-C reflect changes in protein metabolism. The finding that some subjects were in mild negative nitrogen balance during refeeding yet had positive increments in somatomedin-C suggests that somatomedin-C correlates best with acute directional changes in nitrogen balance, rather than total body nitrogen stores. This conclusion is reinforced by the data in Table II which show that during fasting, somatomedin-C falls substantially at a time when nitrogen stores have changed little, and with refeeding for 5 d somatomedin-C returns to 68% of control levels at a time when relatively little total nitrogen repletion has occurred. It is not known whether the changes in somatomedin-C are determining nitrogen balance, or simply reflect changes in this process. Since somatomedin-C stimulates protein synthesis in a variety of tissues (24, 25), it is tempting to speculate that reduction in its serum concentrations could mediate a decrease in the rate of protein synthesis.

Although there is remarkable homogeneity of our subjects' somatomedin-C responses during refeeding, two individuals varied from the typical response pattern. One subject had a minimal somatomedin-C increment when refed the normal diet. This may have been due to increased caloric expenditure secondary to an unauthorized increase in the level of physical activity. In another subject there was evidence of a high basal energy requirement (50 kcal/kg per d to maintain weight). Nitrogen balance can be altered by such variables as age (26), sex (27), basal metabolic rate (28), body temperature (29), previous dietary intake (30), and changing energy expenditure during refeeding (31). Since somatomedin-C appears to be a very sensitive indicator of nutritional status, it is probable that some of these variables can modify the somatomedin-C response to dietary manipulation.

Because dietary manipulation and nutritional status influence secretion of several different hormones, the possibility should be considered that the observed changes in somatomedin-C might be secondary to alterations in other hormones. Insulin levels decline with fasting and rise with refeeding (32, 33). In humans, feeding a diet rich in protein but deficient in energy is accompanied by low levels of insulin (34), suggesting that energy may be the more important determinant of insulin concentrations. Insulin levels correlate with changes in nitrogen balance and play an important role in regulating protein synthesis during nutritional deprivation. The relationship between insulin and somatomedin in humans is less clear. Although somatomedin production is increased by in vitro perfusion of rat liver with insulin (35) there is no in vivo human data to suggest that insulin administration alone, in the absence of appropriate nutrients, can restore serum somatomedin-C concentrations. Although it is possible that in the presence of sufficient nutrients insulin may facilitate somatomedin-C production, it seems doubtful that the dramatic changes in somatomedin-C observed in our subjects are caused by the minimal changes in insulin. In fact, there was no significant correlation between somatomedin-C and insulin during fasting ($r = 0.35$) or refeeding the normal diet ($r = -0.22$). It also appears unlikely that growth hormone is directly involved in the modulation of the changes in somatomedin-C, since the concentrations of these two substances change in opposite directions with fasting and refeeding. Furthermore, starvation causes

growth hormone resistance, manifested by raised serum growth hormone levels (2, 3) low serum somatomedin concentrations (2, 3), an inability of administered growth hormone to raise serum somatomedin (4), and peripheral resistance to the action of growth hormone (4). The increases in cortisol (36) and glucagon (37), which occur with starvation, also seem to be unlikely modulators of somatomedin-C. Although cortisol has been reported to reduce bioassayable somatomedin activity (38, 39), we have observed that it does not affect immunoreactive somatomedin-C (5).

Even though the mechanism(s) for the modulation of somatomedin-C during fasting and refeeding and the relationship of these changes to protein synthesis and utilization remain undefined, our results suggest that somatomedin-C could be an important modulator of nutritional repletion following fasting. They also raise the possibility that measurement of serum concentrations of this growth factor might be useful in monitoring nutritional status of patients suffering from diseases which affect absorption of nutrients or which require nutritional support. Before these applications can be utilized, however, further studies are needed to determine the effect of acute and chronic illness on somatomedin-C concentrations.

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