Effects of Fasting on Gluconeogenesis from Alanine in Nondiabetic Man

J. L. CHIASSON, R. L. ATKINSON, A. D. CHERRINGTON, U. KELLER, B. C. SINCLAIR-SMITH, W. W. LACY, AND J. E. LILJENQUIST

SUMMARY

The present study was designed to determine the effect of fasting on gluconeogenesis from alanine. This process was measured by combining the infusion of ^{14}C-alanine with the A–V difference technique. Normal subjects were studied after 12 and 48 h of fasting and obese subjects were studied after 18 days of therapeutic starvation. After a 12-h fast the hepatic venous ^{14}C-alanine specific activity was 34% lower than the arterial ^{14}C-alanine specific activity, which was consistent with a dynamic exchange of alanine between the intestine and the plasma and with a net release of unlabeled alanine by the intestine. Under these conditions the net splanchnic alanine uptake (106 μmol/min) would underestimate the actual hepatic extraction of alanine, which could be estimated as at least 147 μmol/min. After 48 h and 18 days of fasting, the ^{14}C-alanine specific activity equaled that in the arterial plasma, indicating cessation of intestinal release of alanine into the portal circulation. Under these circumstances, net splanchnic alanine uptake would equal net hepatic alanine extraction. Thus, actual hepatic alanine extraction rates at 48 h and 18 days of fasting were 134 and 94 μmol/min, respectively.

In the face of a decreasing hepatic alanine extraction with fasting, gluconeogenesis from alanine increased by 100% (from 41 to 82 μmol/min) after 48 h of fasting. After 18 days of starvation 51 μmol/min of alanine was converted to glucose, a rate still 25% higher than after a 12-h fast.

We concluded: (a) fasting is associated with a gradual decrease in hepatic alanine extraction; (b) however, gluconeogenesis from alanine is increased after 48 h and 18 days of fasting due to a more efficient intrahepatic conversion of alanine to glucose.

DIABETES 28:56–60, January 1979.

In the postabsorptive state, glycogenolysis is the main source of glucose released by the liver.[1] Because the capacity of the liver to store glycogen is limited, the process of glycogenolysis cannot supply glucose indefinitely during prolonged fasting.[2] Therefore, man's ability to survive after glycogen stores are depleted is dependent in part on the body's ability to derive the necessary glucose via gluconeogenesis. The major gluconeogenic amino acid precursor in the postabsorptive state and during prolonged fasting appears to be alanine.[3]

Felig et al. studied the metabolism of alanine in fasting.[4] These authors noted that the arterial–hepatic venous difference for alanine increased markedly in three subjects fasted 36–48 h as compared with subjects studied in the postabsorptive state. They also noted that in prolonged fasted man (5–6 wk) the net splanchnic alanine uptake was markedly decreased. From these data, it was concluded that gluconeogenesis from alanine was markedly stimulated in early fasting but decreased in prolonged fasting.

While the measurement of alanine uptake by the splanchnic bed is obviously related to the rate of gluconeogenesis from alanine, this measurement fails to take into account the fact that the alanine extracted by the liver may have several intrahepatic fates, only one of which is to be converted to glucose. We reported previously that only approximately 30% of the ^{14}C-alanine extracted by the splanchnic bed in the postabsorptive state in man appeared as ^{14}C-glucose.[5] We also reported that both absolute[5] and relative hyperglucagonemia[6] (decrease in insulin in the face of basal glucagon) can double the amount of ^{14}C-alanine converted to ^{14}C-glucose before having any effect on the amount of alanine extracted by the liver. These hormonal conditions are similar to the pattern observed in starvation in which the plasma glucagon level has been shown to rise and the insulin level to fall.[4] The question thus arises as to whether the body's adaptation to fasting involves an increased efficiency of conversion of alanine into glucose as well as an alteration in the efficiency of alanine extraction by the liver. To investigate these ques-

From the Departments of Medicine and Physiology, Vanderbilt Medical Center, Nashville, Tennessee 37232, and Department of Medicine, Fort Campbell, Kentucky.

Dr. Atkinson's present address is Department of Medicine, University of Virginia Medical School, Charlottesville, Virginia.

Accepted for publication 4 October 1978.

tions the process of gluconeogenesis was studied by using the combined technique of hepatic vein catheterization plus constant ^{14}C-alanine infusion in men fasted 12 and 48 h and 18 days.

MATERIALS AND PREPARATIONS

Sterile, pyrogen-free ^{14}C-L-alanine (256 μCi/mmol) (New England Nuclear, Boston, Massachusetts) was used to follow the conversion of alanine to glucose. Hepatic blood and plasma flows were determined by using indocyanine green from Hynson, Westcott, and Dunning (Baltimore, Maryland). Phadebas Insulin Radioimmunoassay Kit was purchased from Pharmacia Fine Chemicals (Piscataway, New Jersey), and Trasylol from FBA Pharmaceuticals (New New, New York). Glucagon 30K antiserum was purchased from the University of Texas Southwestern Medical School (Dallas, Texas), and standard glucagon and glucagon-^{125}I from Novo Alle (Copenhagen, Denmark).

The ^{14}C-alanine was mixed with saline containing 1–2 ml of the subject's plasma and was infused at a rate varying between 0.23 and 0.68 μCi/min for a total dose not exceeding 200 μCi. Three milliliters of plasma were added to freshly prepared indocyanine green to increase its stability.[7]

SUBJECTS

Seventeen normal male volunteers and three obese subjects participated in this study (Table 1). None of the subjects had any personal history of diabetes mellitus or any other endocrine or major disease. Each subject had a normal 3-h oral glucose tolerance test (40 g of glucose per square meter of body surface).[8] Hepatic function was also normal as assessed by bromsulfthalein extraction, serum bilirubin, and alkaline phosphatase. Each subject had a normal serum urea nitrogen and urinalysis, and cardiovascular function was normal by history, physical examination, chest X-ray photograph, and electrocardiogram. Complete blood counts including a sedimentation rate were normal in all subjects.

PROCEDURES

The subjects were studied after a 12-h (N = 8), after a 48-h (N = 9), or after an 18-day fast (N = 3). As each subject received 200 μCi of ^{14}C-alanine by constant infusion, he could be studied on only one occasion. This necessitated that the 12- and 48-h fasted groups be composed of different subjects. The three obese subjects were at least 20% over ideal body weight and were fasted for 18 days for therapeutic weight reduction. They were hospitalized at the Fort Campbell Army Hospital during this prolonged fast and kept under close supervision. They each received a daily multivitamin and 8 oz of broth containing electrolytes and approximately 16 calories. On the day before the study, they were transferred to the Vanderbilt Hospital Clinical Research Center.

The nature, purpose, and possible risks of the procedures were fully explained to each subject before his voluntary consent was obtained. The protocol for the present study was reviewed and approved by the Vanderbilt University Clinical Investigation Committee, which has the responsibility for protecting the rights of human volunteers participating in research studies.

All studies were performed in the Vanderbilt University Hospital Cardiac Catheterization Laboratory. A Teflon catheter was inserted percutaneously in the left brachial artery. A small 1-cm cutdown was then performed on the contralateral arm over a small tributary vein distal to the right antecubital fossa. A no. 8F Cournand catheter was guided through this vein to a rightsided hepatic vein and under fluoroscopy was positioned 3–4 cm from the wedge position. Patency of the hepatic vein catheter was maintained by a continuous saline infusion without added anticoagulant.

Each subject received a trace amount of ^{14}C-alanine given as constant infusion through a peripheral vein from 0–80 min. The first 40 min of the study was allotted for equilibration of the tracer and was followed by a 40-min study period (40–80 min).

Indocyanine green was also infused throughout the study for determination of hepatic blood and plasma flows. Blood samples were drawn simultaneously from the brachial artery and hepatic veins at 5- or 10-min intervals. Less than 500 ml of blood was withdrawn during the study, the volume of which was replaced with saline infused at 5–7 ml/min. All subjects were admitted to the Clinical Research Center on the day before the study and were kept under observation until the morning after the study, before discharge.

ANALYTICAL METHODS

Plasma alanine and ^{14}C-alanine were determined by a rapid short-column chromatography technique.[5] To measure plasma ^{14}C-glucose, plasma was deproteinized using the Somogyi-Nelson technique and passed on ion exchange resins.[5] The radioactivity in alanine and glucose was determined in a liquid scintillation spectrometer.

Whole blood glucose was measured within 4 min by the Hoffman ferricyanide reaction, using the Technicon Autoanalyzer.[9] Unger's 30K antiserum was used to assay

TABLE 1
Clinical data on subjects

Subjects	Age (yr)	Height (cm)	Weight (kg)	Surface area (m^2)	Estimated hepatic plasma flow (ml/min)
12-h fast (N = 8)					
Mean ± SE	26.0 ± 2.0	182.0 ± 2.0	79.0 ± 4.0	1.99 ± 0.04	1034 ± 58
48-h fast (N = 9)					
Mean ± SE	24.0 ± 2.0	177.0 ± 2.0	75.0 ± 3.0	1.92 ± 0.05	1138 ± 126
18-day fast (N = 3)					
Mean ± SE	25.0 ± 4.0	175.0 ± 2.0	118.0 ± 9.0	2.31 ± 0.09	1042 ± 136

TABLE 2
Effect of fasting on arterial glucose concentration and net splanchnic glucose production

Subjects	Arterial glucose concentration* (mg/dl)	Net splanchnic glucose production* (mg/min)
12-h fast (N = 8)		
Mean ± SE	93 ± 2	153 ± 10
48-h fast (N = 9)		
Mean ± SE	69 ± 3†	72 ± 9†
18-day fast (N = 3)		
Mean ± SE	75 ± 5‡	41 ± 13‡

* Mean ± SE of the average (mean of five determinations at 10-min intervals) of each individual within the group.
† $P < 0.01$ as compared with 12-h fasted group.
‡ $P < 0.02$ as compared with 12-h fasted group.

immunoreactive glucagon, and dextran-coated charcoal was used to separate the unbound from the bound glucagon.[10] Immunoreactive insulin was assayed by using the Sephadex bound antibody procedure.[11,12] The plasma concentrations of indocyanine green were determined in a Beckman spectrophotometer at 805–815 mμ and calculated according to the method of Leevy et al.[13]

The conversion of alanine to glucose was calculated by dividing the net splanchnic ^{14}C-glucose production (cpm/min) by the hepatic venous plasma alanine-^{14}C specific activity (cpm/μmol).[14] Statistical significance was determined by using the Student's *t* test or paired *t* test whenever applicable.[15]

RESULTS

Glucagon, insulin, and estimated hepatic plasma and blood flow. Mean basal immunoreactive glucagon was 102 ± 7 pg/ml at 12 h of fasting, 175 ± 7 pg/ml in a second group of subjects fasted 48 h, and 157 ± 17 pg/ml in three obese subjects after an 18-day fast, still significantly higher than in the subjects studied in the postabsorptive state. Mean basal immunoreactive insulin levels were 8 ± 1, 8 ± 1, and 7 ± 1 μU/ml in the three different groups of subjects, respectively.

The mean estimated hepatic plasma flow for each group is shown in Table 1. The mean estimated hepatic plasma and blood flows for all subjects were 1138 ± 28 ml/min, and 1913 ± 49 ml/min, respectively.

Arterial glucose concentration and net splanchnic glucose production. After an overnight fast the mean arterial glucose concentration was 93 ± 2 mg/dl. In the 48-h fasted subjects, it was 69 ± 3 mg/dl and 75 ± 5 mg/dl in the 18-day fasted subjects (Table 2). Also shown in Table 2 is the net splanchnic glucose production (NSGP) rate. There was a marked decline in NSGP from 153 ± 10 mg/min after 12 h of fasting to 72 ± 9 mg/min in the 48-h fasted subjects. In the 18-day fasted subjects, NSGP was 41 ± 13 mg/min.

Arterial plasma alanine concentration, net splanchnic alanine uptake, and alanine specific activity. Mean arterial plasma alanine concentration was 249 ± 15 μmol/L after an overnight fast and decreased to 170 ± 9 and 161 ± 23 μmol/L at 48 h and 18 days of fasting, respectively (Table 3). In the postabsorptive state the net splanchnic extraction of alanine was 106 ± 12 μmol/min, while it was 134 ± 14 μmol/min after a fast of 48 h and 94 ± 19 μmol/min after 18 days of fasting.

The plasma alanine specific activity measured in the artery and in the hepatic vein remained relatively constant throughout the study period in each fasting state. After 12 h of fasting, the hepatic vein ^{14}C-alanine specific activity was consistently lower than the arterial ^{14}C-alanine specific activity at a ratio of 0.66 ± 0.03, which was constant over the study period. However, in the 48-h and 18-day fasted groups the hepatic vein and arterial ^{14}C-alanine specific activity were similar, resulting in ratios of 1.00 ± 0.04 and 0.99 ± 0.01, respectively, again remaining constant over the study period (Figure 1).

Arterial plasma alanine converted to glucose by the liver. In the postabsorptive state, 41 ± 4 μmol of circulating alanine were converted to glucose per minute and released by the liver (Table 4). This conversion rate increased by 100% (82 ± 8 μmol/min) after 48 h of fasting. After 18 days of fasting the conversion rate was 51 ± 9 μmol/min. In the 12-h fasted group, 39 ± 6% of the alanine extracted by the splanchnic bed was directly converted to glucose. The fractional conversion rate of the extracted alanine was increased to 61 ± 4% and 54 ± 7% after 48 h and 18 days of fasting, respectively.

TABLE 3
Effect of fasting on arterial plasma alanine concentration and net splanchnic alanine uptake

Subjects	Arterial alanine concentration* (μmol/L)	Net splanchnic alanine uptake* (μmol/min)
12-h fast (N = 8)		
Mean ± SE	249 ± 15	106 ± 12
48-h fast (N = 9)		
Mean ± SE	170 ± 9†	134 ± 14
18-day fast (N = 3)		
Mean ± SE	161 ± 23‡	94 ± 19

* Mean ± SE of the average (mean of five determinations at 10-min intervals) of each individual within the group.
† $P < 0.01$ as compared with 12-h fasted group.
‡ $P < 0.02$ as compared with 12-h fasted group.

DISCUSSION

In the present study we examined the effect of fasting for 12 h, 48 h, and 18 days on gluconeogenesis from alanine in nondiabetic man. This process of new glucose formation was assessed by measuring both the hepatic extraction of alanine and its conversion to glucose by the liver after extraction. Our data support the hypothesis that, as fasting is prolonged, the liver becomes much more efficient in the utilization of alanine for new glucose formation.

In 1969, Felig et al. reported three subjects who, after a 36–48-h fast underwent hepatic vein catheterization for diagnostic purposes.[4] The arterial–hepatic venous alanine differences were markedly increased as compared with those in postabsorptive subjects, leading these authors to suggest that hepatic alanine extraction was increased and, therefore, that gluconeogenesis was enhanced. These same authors have shown that in postabsorptive man the intestine adds alanine directly into the splanchnic circulation.[16,17] These observations thus indicate that in the postabsorptive state measurement of the net splanchnic alanine uptake would underestimate the actual extraction of alanine by the liver.

In our study, we did not measure the alanine concentration in the portal vein. We did measure, however, the ^{14}C-alanine specific activity in the hepatic vein as well as in the brachial artery. The dilution of the ^{14}C-alanine specific activity in the hepatic vein provides us with an index of the relative amount of unlabeled alanine added to the plasma as it traverses the splanchnic bed. We expressed this dilution as the ratio of hepatic venous to the arterial ^{14}C-alanine specific activity. In our 12-h fasted subjects, this ratio was 0.66 ± 0.03, indicating a 34% dilution throughout the splanchnic bed. Without portal vein alanine concentration and specific activity, neither the site nor the cause for this 34% dilution of the ^{14}C-alanine specific activity across the splanchnic bed can be deduced. The dilution could have occurred either across the intestine or at the level of the liver. The cause of the dilution could have been secondary to net addition of cold alanine, to flux of alanine between the plasma and extravascular pools (without net addition), or, finally, to a combination of the two. In studies from our laboratory (Shulman et al., manuscript in preparation), we observed that this dilution occurs totally within the intestine (the liver itself not affecting the ^{14}C-alanine specific activity) and that it is due partially to net addition of cold alanine and partially to exchange. Although in the present study we are unable to determine how much of the observed dilution was due to net addition by the intestine, the data published by Felig[16,17] would indicate that a minimum of 41 μmol/min (calculated as product of arterioportal alanine differences × flow in two separate studies) was due to net addition. This would suggest that in our 12-h fasted subjects, at least 147 μmol of alanine were extracted by the liver per minute.

At 48 h and 18 days of fasting, the net splanchnic alanine uptake was 134 and 94 μmol/min, respectively (Table 3). To our knowledge there has been no arterioportal alanine differences measured in man at 48 h of fasting or after prolonged starvation. But here also, the dilution of the ^{14}C-alanine specific activity as it traverses the splanchnic bed gives us some invaluable insight concerning the alanine flux across the intestine and the liver. Interestingly, the hepatic venous:arterial ^{14}C-alanine specific activity ratio was 1.00 ± 0.04 at 48 h of fasting and 0.99 ± 0.01 after 18 days of fasting (Figure 1). This means that there was no dilution of the ^{14}C-alanine specific activity within the splanchnic bed, indicating that there was no net addition of unlabeled alanine by the intestine. Under these conditions, the net splanchnic alanine uptake would essentially be equal to the actual hepatic alanine extraction. Thus, our data would suggest that the net hepatic alanine uptake decreases progressively as fasting is prolonged: a minimum of 147 μmol/min after an overnight fast, 134 μmol/min after a 48-h fast, and 94 μmol/min after 18 days of fasting. Had we limited our observation to this parameter as an index of gluconeogenesis, we would have concluded erroneously that gluconeogenesis decreased progressively with fasting.

It was because of this obvious limitation that we also measured the conversion of circulating ^{14}C-alanine to glucose by the liver. This conversion is calculated by dividing the net splanchnic ^{14}C-glucose production by the ^{14}C-alanine specific activity. Since the arterial ^{14}C-alanine specific activity can be diluted by unlabeled alanine released by the intestine, the hepatic vein ^{14}C-alanine specific activity is more representative of the specific activity seen

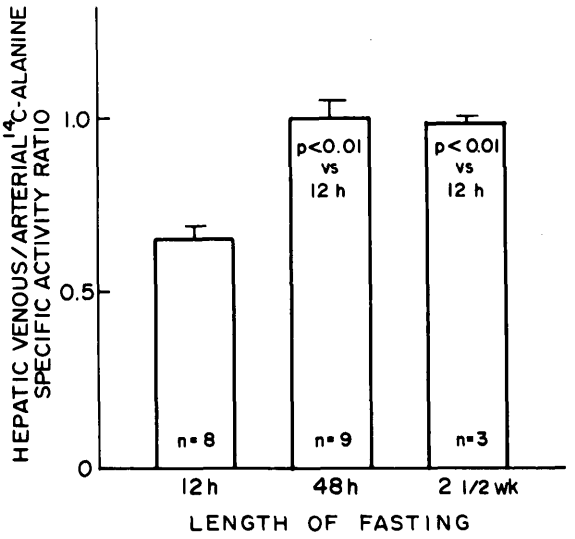

FIGURE 1. The hepatic venous/arterial ratio of ^{14}C-alanine specific activity in 12-h, 48-h, and 2½-wk fasted man.

by the hepatocyte. This specific activity was therefore used to calculate the conversion of alanine to glucose. The limitation of this technique has already been discussed in a recent review.[14] In our study, the amount of ^{14}C-alanine appearing in ^{14}C-glucose was increased by 100% after 48 h of fasting ($P < 0.01$) and by 25% after 18 days of fasting ($P > 0.05$) when compared with the amount appearing in the postabsorptive state. This increase in appearance of ^{14}C label in glucose could be due to two possible mechanisms: (a) an actual stimulation of the gluconeogenic pathway with shunting of ^{14}C-alanine into ^{14}C-glucose formation; or, (b) a decreased dilution of the ^{14}C-labeled precursor pool within the hepatocyte. The two major intrahepatic sources of unlabeled carbon skeletons entering this precursor pool are hepatic proteolysis and glycolysis. Hepatic proteolysis is increased in fasting,[18] which would lead to decreased ^{14}C-specific activity of the precursor pool, thus causing the method to underestimate the conversion of alanine to glucose. Glycolysis, on the other hand, is decreased in fasting[19] and therefore would cause the method to err in the opposite direction. In in vivo studies, it is not possible at present to quantify the effect of decreased glycolysis or increased proteolysis on the appearance of ^{14}C label in glucose. Under the conditions of the

TABLE 4
Effect of fasting on the conversion of arterial plasma alanine to glucose and percent of net splanchnic alanine uptake converted to glucose

Subjects	Alanine converted to glucose* (μmol/min)	Extracted alanine converted to glucose* (% of NSAU†)
12-h fast (N = 8)		
Mean ± SE	41 ± 4	39 ± 6
48-h fast (N = 9)		
Mean ± SE	82 ± 8‡	61 ± 4§
18-day fast (N = 3)		
Mean ± SE	51 ± 9	54 ± 7

* Mean ± SE of the average (mean of five determinations at 10-min intervals) of each individual within the group.
† Net splanchnic alanine uptake.
‡ $P < 0.01$ as compared with 12-h fasted group.
§ $P < 0.02$ as compared with 12-h fasted group.

present study, however, these two factors would tend to negate each other. Our data would indicate, therefore, that after 48 h of fasting gluconeogenesis from alanine has doubled when compared with that present in the overnight fasted subject. In the 18-day fasted subjects, the conversion of alanine to glucose was 25% higher than in the overnight fasted groups but was not significant. The lack of significance could be due to the small number studied (N = 3) or to the fact that gluconeogenesis from alanine had decreased to rates comparable with those observed after an overnight fast, possibly because of decreased availability of substrate. But no matter which, it becomes evident that despite a decrease in net hepatic alanine uptake as fasting is prolonged, gluconeogenesis from alanine is increased at 48 h of fasting and is still well maintained and possibly even increased at 18 days of fasting when compared with the postabsorptive state.

As fasting was prolonged, the arterial alanine concentration fell markedly (Table 3), a finding consistent with observations of others.[4,20] Various investigators reported that skeletal muscle alanine release is increased in early starvation, thus providing increased amounts of alanine for hepatic extraction.[21–23] Since hepatic alanine extraction does not appear to increase after 48 h of fasting the excess alanine released from skeletal muscle may be offset by the cessation of intestinal alanine release. As arterial alanine levels decreased markedly between 12 and 48 h of fasting (Table 3), the decrease in net intestinal alanine release may be of greater magnitude than the increase in net alanine release by skeletal muscle. However, although the actual hepatic alanine extraction decreased with fasting, the fractional hepatic extraction of alanine increased. Thus, with fasting, the liver was more efficient in extracting alanine from plasma, but was not extracting more alanine in an absolute sense. The only way in which gluconeogenesis from alanine could be increased under these conditions would be if intrahepatic conversion of alanine to glucose were increased. Our data are compatible with such a hypothesis. Despite a decrease in the amount of alanine extracted by the liver, the process of gluconeogenesis had become markedly stimulated by an increased intrahepatic conversion of the extracted alanine into glucose. As net splanchnic glucose production had fallen 50% by 48 h of fasting and 75% by 18 days of fasting, the contribution of circulating alanine to glucose production was increased markedly.

The mechanism by which the liver becomes more efficient in extracting alanine and converting alanine to glucose cannot be ascertained by the present study. It seems likely, however, that the hormonal changes accompanying fasting may play a key role in determining both the extraction of alanine by the liver and the intrahepatic fate of the extracted alanine. The fasting state is characterized by a fall in plasma insulin and a rise in plasma glucagon.[4] In our subjects, for reasons not clear, we did not see a significant fall in plasma insulin levels. The plasma glucagon levels, however, clearly rose significantly. It is therefore possible that glucagon excess as well as insulin lack could be responsible for these observations.[5,6]

In conclusion, the data from the present study indicate that (a) fasting is associated with a gradual decrease in the absolute amount of alanine extracted by the liver although the fractional uptake of alanine is increased; (b) gluconeogenesis from alanine is substantially increased at 48 h and slightly increased at 18 days of fasting due to a more efficient intrahepatic conversion of alanine to glucose; and (c) there is a dynamic exchange of alanine between the intestine and the plasma with net addition of alanine to the plasma; this release of alanine by the intestine appears to cease as fasting is prolonged.

ACKNOWLEDGMENTS

The investigation was supported by NIH grants AM-19587 and AM-18243, the Vanderbilt University Diabetes-Endocrinology Research Center grant AM-17026, and the General Clinical Research Center grant 5M01RR95.

REFERENCES

[1] Felig, P., and Wahren, J.: Influence of endogenous insulin on splanchnic glucose and amino acid metabolism in man. J. Clin. Invest. *50*:1702–11, 1971.

[2] Hultman, E., and Nelsson, L. H.: Liver glycogen in man. Effect of different diets and muscular exercise. *In*: Muscle Metabolism During Exercise. Pernow, B., and Saltin, B., Eds. New York, Plenum Press, 1971, p. 143.

[3] Felig, P., Pozefsky, T., Marliss, E., and Cahill, G. F.: Alanine: key role in gluconeogenesis. Science *167*:1003–4, 1970.

[4] Felig, P., Owen, O. E., Wahren, J., and Cahill, G. F., Jr.: Amino acid metabolism during prolonged starvation. J. Clin. Invest. *48*:584–94, 1969.

[5] Chiasson, J. L., Liljenquist, J. E., Sinclair-Smith, B. C., and Lacy, W. W.: Gluconeogenesis from alanine in normal postabsorptive man. Intrahepatic stimulatory effect of glucagon. Diabetes *24*:574–84, 1975.

[6] Jennings, A. S., Cherrington, A. D., Liljenquist, J. E., Keller, U., Lacy, W. W., and Chiasson, J. L.: The roles of insulin and glucagon in the regulation of gluconeogenesis in the postabsorptive dog. Diabetes *26*:841–56, 1977.

[7] Leevy, C. M., and Bender, J.: Physiology of dye extraction by the liver: comparative studies of sulfobromophthalmin and indocyanine green. Ann. N. Y. Acad. Sci. *111*:161, 1973.

[8] Klimt, C. R., Prout, T. E., Bradley, R. F., Dolger, H., Fisher, G., Gastineau, C. F., Marks, H., Meinert, C. L., and Schumacher, O. P.: Standardization of the oral glucose tolerance test. Diabetes *18*:299, 1969.

[9] Technicon Laboratory. Method File N-2A Technicon Instrument Corporation, Chauncey, New York.

[10] Aguilar-Parada, E., Eisentraut, A. M., and Unger, R. H.: Pancreatic glucagon secretion in normal and diabetic subjects. Am. J. Med. Sci. *257*: 415–19, 1969.

[11] Yalow, R. S., and Berson, S. A.: Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. *39*:1157, 1960.

[12] Wide, L., and Porath, J.: Radioimmunoassay of proteins with the use of sephadex coupled antibodies. Biochem. Biophys. Acta *130*: 257–60, 1966.

[13] Leevy, C. M., Mendelhall, C. L., Lesko, W., and Howard, M. M.: Estimation of hepatic blood flow with indocyanine green. J. Clin. Invest. *41*:1169–79, 1962.

[14] Chiasson, J. L., Liljenquist, J. E., Cherrington, A. D., Jennings, A. S., and Lacy, W. W.: Gluconeogenesis: methodological approaches in vivo. Fed. Proc. *36*:229–35, 1977.

[15] Snedecor, G. W.: Statistical Methods, 5th edit. Ames, Iowa, Iowa State University Press, 1965, p. 35.

[16] Felig, P., Wahren, J., and Raf, L.: Evidence for intraorgan amino acid transport by blood cells in humans. Proc. Nat. Acad. Sci. (USA) *70*:1775–79, 1973.

[17] Felig, P., and Wahren, J.: Amino acid metabolism in exercising man. J. Clin. Invest. *50*:2703–14, 1971.

[18] Addis, T., Poo, L. J., and Lew, W.: Protein loss from liver during 2-day fast. J. Biol. Chem. *115*:117–18, 1936.

[19] Klain, G. J., and Burlington, R. F.: Effect of cold on glucose metabolism of fasted and fasted-refed rats. Am. J. Physiol. *213*:209–14, 1967.

[20] Wolfe, B. M., Havel, J. R., Marliss, E. B., Kane, J. P., Seymour, J., Ahuja, S. P.: Effects of a 3-day fast and of ethanol on splanchnic metabolism of FFA, amino acids and carbohydrates in healthy young men. J. Clin. Invest. *57*:329–40, 1976.

[21] Ruderman, N. B., and Berger, M.: The formation of alanine and glutamine in skeletal muscle. J. Biol. Chem. *249*:5500–506, 1974.

[22] Odessey, R., Khairallah, E. A., and Goldberg, A. L.: Origin and possible significance of alanine production by skeletal muscle. J. Biol. Chem. *249*:7623–29, 1974.

[23] Pozefsky, T., Tancredi, R. G., Moxby, R. T., Dupre, J., and Tobin, J. D.: Effects of brief starvation on muscle amino acid metabolism in nonobese man. J. Clin. Invest. *57*:444–59, 1976.