The Glucose-Alanine Cycle

By Philip Felig

Alanine is quantitatively the primary amino acid released by muscle and extracted by the splanchnic bed in postabsorptive as well as prolonged fasted man. The hepatic capacity for conversion of alanine to glucose exceeds that of all other amino acids. Insulin inhibits gluconeogenesis by reducing hepatic alanine uptake. In contrast, in diabetes, an increase in hepatic alanine extraction is observed in the face of diminished circulating substrate. In prolonged fasting, diminished alanine release is the mechanism whereby gluconeogenesis is reduced. In circumstances in which alanine is deficient, such as pregnancy and ketotic hypoglycemia of infancy, fasting hypoglycemia is accentuated. Augmented glucose utilization in exercise and hyperpyruvicemia consequent to inborn enzymatic defects are accompanied by increased circulating levels of alanine. These data thus suggest the existence of a glucose-alanine cycle in which alanine is formed peripherally by transamination of glucose-derived pyruvate and transported to the liver where its carbon skeleton is reconverted to glucose. The rate of recycling of glucose carbon skeletons in this pathway appears to occur at approximately 50% of that observed for the Cori (lactate) cycle.

IN RECENT YEARS studies from a number of laboratories have focused attention on the role of substrate in the regulation of gluconeogenesis.[1-5] Particular emphasis has been placed on the central role of alanine as the key protein-derived gluconeogenic precursor.[6] In addition, based on a variety of observations to be discussed below, the thesis has been advanced that alanine released from extrahepatic tissues consists not only of preformed alanine derived by catabolism of cellular proteins, but also includes peripherally synthesized alanine formed by in situ transamination of glucose-derived pyruvate. This postulated sequence of events has been described by Mallette et al.[4] and by Felig et al.[6] as the alanine cycle (Fig. 1). It is the purpose of this review to examine in detail the metabolism of alanine, particularly as it relates to glucose homeostasis. Furthermore, in view of the intimate relation between the metabolism of alanine and glucose as both precursor and product, it is felt that the cycle is more aptly described as the "glucose–alanine cycle."[7]

AMINO ACID FLUX IN POSTABSORPTIVE STATE

The postabsorptive or overnight fasted condition is generally considered a steady state with regard to glucose homeostasis. Thus, under normal circum-

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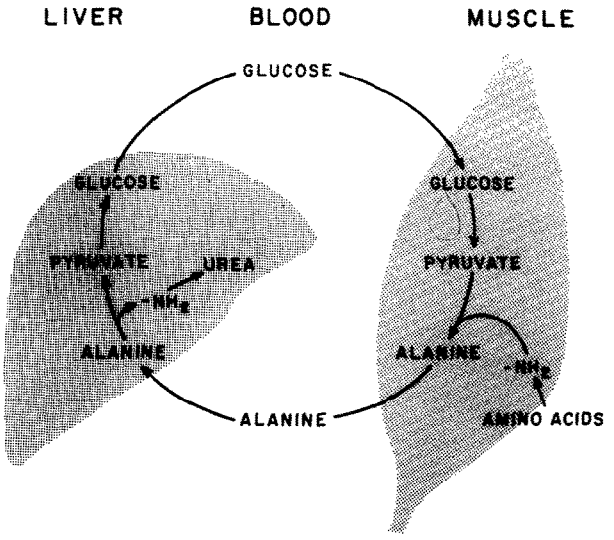

Fig. 1. The glucose-alanine cycle. Glucose released by the liver is taken up by muscle where it is converted to pyruvate and transaminated to form alanine. The alanine thus synthesized is released by muscle and taken up by the liver where its carbon skeleton is reconverted to glucose thus completing the cycle.

stances, circulating glucose levels show little fluctuation as hepatic glucose production keeps pace with peripheral glucose utilization.[8] In contrast, body fat stores are in a state of negative balance with free fatty acids being released from stored triglyceride in adipose tissue to meet the energy demands of muscle, liver, and kidney. A situation analogous to that of adipose tissue exists with respect to protein.

Following an overnight fast, muscle, the primary repository of body protein, is in a state of negative nitrogen balance, releasing free amino acids. Van Slyke and Meyer were the first to note an increase in the content of free amino acids in the tissues of starved dogs and concluded that catabolism of tissue proteins is the source of circulating amino acids in the fasted condition.[9] More direct evidence that muscle releases amino acids in the postabsorptive state was provided by London et al., who studied the arterio-venous differences for free amino acids across the deep venous bed of the human forearm.[10] In these studies, consistently negative arterio-venous differences were noted for 11 of 17 amino acids. Although not commented upon by these authors, alanine release exceeded that of all other amino acids, accounting for 30% of total amino acid output.[10] More recent studies (Fig. 2) have confirmed the primacy of alanine in the net flux of amino acids from peripheral protein stores, both in the postabsorptive state,[11,12] as well as after a prolonged fast.[6] The importance of alanine as a vehicle for the transport of amino nitrogen is further underscored by the observation that its output accounts for more than 80% of the total amino acids released by the human myocardium.[13,14]

In attempting to account for the pattern of amino acid output from muscle in the basal state, the following must be taken into consideration: (1) alanine comprises no more than 7%–10% of the amino acid residues in skeletal[15] and cardiac muscle proteins;[16] (2) a specific polyalanyl protein has not been identified in muscle; and (3) were such a protein in fact present in basal man in small and consequently undetectable amounts, it would not account for

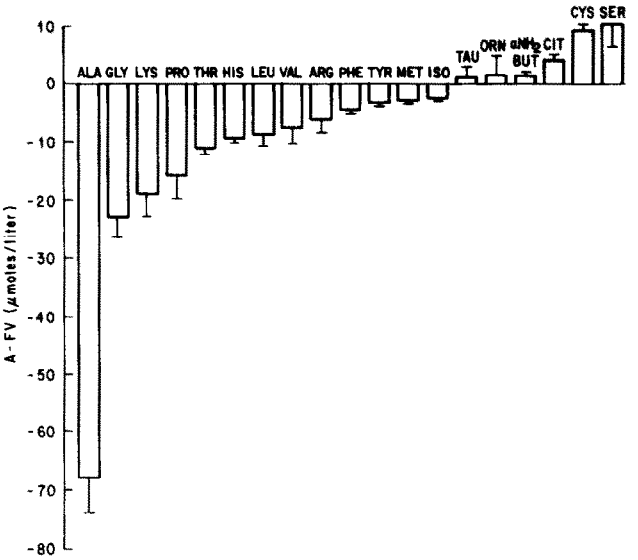

Fig. 2. Arterio-femoral venous differences (A-FV) of individual plasma amino acids in normal postabsorptive man. Negative values indicate a net output which was greatest for alanine. (By permission.[12])

the persistence of the relative preeminence of alanine output after 5–6 wk of starvation.[6] It is thus apparent that release of preformed alanine from muscle protein or from the intracellular amino acid pool cannot explain the predominant contribution of this amino acid to total nitrogen release from muscle. Consequently, it has been suggested that alanine is synthesized de novo in muscle by transamination of pyruvate.[6] Supporting this conclusion is the direct linear correlation between circulating concentrations of alanine and pyruvate in basal man;[12] such a relation with pyruvate is not demonstrable for any other amino acid. In addition, in situations of augmented pyruvate availability, such as muscular exercise[12] and chronic hyperpyruvicemia associated with inborn enzymatic defects[17,18] (see discussion below), augmented release and/or accumulation of alanine is demonstrable. While the studies in exercise suggest that the pyruvate utilized in peripheral alanine synthesis is glucose-derived,[12] the possibility must also be considered that the deaminated carbon skeletons of a variety of amino acids are converted in situ to pyruvate and then transaminated to alanine prior to their release from muscle tissue. In such a sequence of events, alanine would not only contribute to the recycling of glycolytic intermediates, but would also provide carbon skeletons for de novo glucose synthesis.

Irrespective of the origin of the pyruvate utilized in the formation of alanine, synthesis of this amino acid in muscle requires an appropriate source of amino groups. In this respect it is noteworthy that the branched chain amino acids (valine, leucine, and isoleucine) are preferentially catabolized in muscle[19,20] rather than liver[19,21] and serve as a ready source of nitrogen for transamination of pyruvate. Significant rates of extrahepatic catabolism have also been demonstrated for glycine, aspartate, and glutamate.[20] Furthermore, a variety of tissues have been shown to utilize alanine synthesis as the mechanism of disposal of nitrogen derived from exogenous loads of virtually all amino acids.[22]

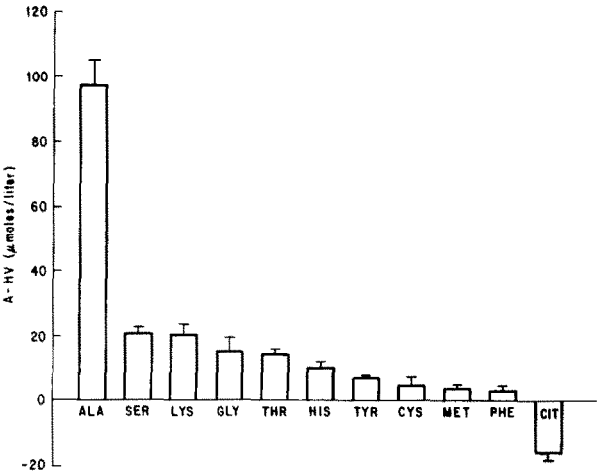

Fig. 3. Arterio-hepatic venous differences (A-HV) of individual plasma amino acids in normal postabsorptive subjects. Only those amino acids for which a significant net exchange across the splanchnic bed was demonstrable, are shown. (By permission.[12])

Since most amino acids do not accumulate in plasma during brief periods of fasting, their output from muscle must be accompanied by some extramuscular site of uptake. That the liver plays a central role in regulating amino acid utilization has been evident since the classical studies of Bollman et al. in hepatectomized dogs.[23] Introduction of the liver perfusion technique by of amino groups. In this respect it is noteworthy that the branched chain amino and of their conversion to glucose.[4] The pattern of extraction of individual amino acids by the splanchnic bed was first investigated in man by Onen et al.[25] Using a semiquantitative paper chromatographic technique, these authors noted that the uptake of alanine exceeded that of all other amino acids.[25] Similar findings have been reported in subsequent studies utilizing the quantitative column chromatographic procedure.[2,26] Of the total amino acid uptake by the splanchnic bed, alanine accounts for approximately 50% (Fig. 3). Particularly noteworthy is the fact that the pattern of splanchnic amino acid uptake conforms quite well to that of peripheral amino acid release (Fig. 2). A notable exception is serine, which is extracted by both liver and muscle, but which is consistently released by the kidney.[27]

ALANINE AND GLUCONEOGENESIS

These observations on splanchnic and peripheral exchange of amino acids clearly implicate alanine as the primary gluconeogenic precursor participating in the flux of protein-derived substrate between muscle and the splanchnic tissues. Two major questions regarding the role of alanine in gluconeogenesis may, however, be raised. (1) Is the liver rather than the gastrointestinal tissues the site of splanchnic alanine uptake? (2) If alanine is in fact extracted by the liver, is conversion to glucose its ultimate fate? With regard to the first question, observations on the arterial-portal venous differences for alanine in humans[12] as well as dogs[28] have revealed a small but consistent net output of this amino acid from the gastrointestinal tissues drained by the portal vein. Thus, the uptake of alanine demonstrable by the hepatic venous catheter technique[2,26] represents an underestimate rather than an overestimate of the true hepatic consumption.

With regard to the second question, studies with isotopically labeled alanine in postabsorptive man have revealed prompt incorporation of this amino acid into blood glucose, with maximum recovery occurring in 30–60 min.[5,29,30] Furthermore, the proportion of the injected dose of alanine recoverable as glucose[30] is comparable to that observed after injection of lactate.[31]

Additional data implicating alanine as a key gluconeogenic precursor are available from in vitro studies with the perfused liver. Ross et al. noted that the rate of glucose production from alanine was among the highest reported for any amino acid.[1] In addition, maximal rates of glucose production from a mixture of amino acids are achieved at three times the normal plasma concentration, whereas maximal gluconeogenic rates from alanine are not achieved until a concentration of 9 m*M* (or 20–30 times the normal level) is reached.[4] That oxidation accounts for a very small proportion of the hepatic uptake of this amino acid is indicated by the observation that the ratio of alanine oxidized to CO_2 to alanine converted to glucose remains constant at 0.13:1.0 over a wide range of concentrations of alanine in the perfusion medium.[32] Finally, the possibility that protein synthesis could account for the predominance of alanine in hepatic amino acid consumption is unlikely, since a polyanalyl protein has not been identified in liver tissue.[33]

The influence of hepatic uptake of alanine on gluconeogenesis may not be restricted to the provision of precursor substrate, but may also involve modification of enzyme activity. An inhibitory effect of alanine on liver pyruvate kinase activity has been reported.[34] Inhibition of this key glycolytic enzyme would tend to promote the utilization of glucose precursors along the gluconeogenic pathway (reversal of glycolysis), since conversion of phosphoenolpyruvate to pyruvate would be blocked. Interestingly, in muscle tissue in which such a function would not be applicable, alanine fails to inhibit pyruvate kinase.[34,35]

INSULIN AND ALANINE METABOLISM

More than 40 yr ago, Luck et al. demonstrated that insulin reduced the amino acid content of blood in a variety of species.[36] Based on observations in eviscerated animals in which insulin prevented a rise in alpha amino nitrogen concentration, Mirsky[37] and Russell[38] suggested that the hypoaminoacidemic effect of insulin was due to stimulation of muscle uptake of amino acids for protein synthesis. Subsequent studies with isotopically labeled amino acids provided direct evidence that insulin enhanced the uptake and incorporation of amino acids into muscle protein.[39] The hypoaminoacidemic effect of insulin was also suggested as the basis of its regulatory action in gluconeogenesis, inasmuch as early studies failed to demonstrate a direct effect of insulin on hepatic glucose production.[40] Examination of the effect of insulin on the circulating levels of individual amino acids, however, casts doubt on the latter hypothesis, and provides further evidence of the uniqueness of alanine metabolism .

In humans as well as experimental animals, the reduction in total plasma amino acid content produced by administration of exogenous insulin,[41,42,43,44] or by stimulation of endogeous insulin secretion by intravenous[45,46] or oral

glucose[47,48] most consistently involves the branched chain amino acids (valine, leucine, and isoleucine), as well as tyrosine and phenylalaline. Although the effect of insulin is in general less marked with respect to the nonessential amino acids,[49] alanine is unique in being the only amino acid for which a consistent decline has not been observed in any of the above studies. Likewise, infusion of insulin into the deep tissues of the human forearm fails to significantly inhibit muscle output of alanine.[11,50] Furthermore, in some studies a tendency toward an elevation in plasma alanine levels has been observed after systemic administration of insulin[42] or glucose.[45,46] Additional evidence that the behavior of alanine vis a vis insulin differs from that of other amino acids is provided by in vitro studies with the isolated rat diaphragm.[51,52] Sinex et al. noted that whereas insulin stimulated incorporation of ^{14}C-alanine into diaphragm protein, this action was inhibited by glucose and pyruvate.[51] In marked contrast, glucose or pyruvate failed to diminish the stimulatory action of insulin on the incorporation into protein of ^{14}C from a variety of other isotopically labeled amino acids (glycine, leucine, isoleucine, phenylalanine, serine, lysine, arginine, glutamate, aspartate, and methionine).[52] Thus, both with respect to the action of insulin in lowering the concentration of plasma amino acids as well as its effect on amino acid incorporation into protein, the behavior of alanine clearly differs from that of other amino acids. A primary resistance on the part of alanine to the effects of insulin on muscle seems unlikely, inasmuch as under appropriate circumstances increased incorporation into protein[51] as well as augmented intracellular accumulation of this amino acid[53,54] have been demonstrated. A more likely explanation for these peculiarities is that in contrast to its action on the metabolism of other amino acids, insulin stimulates peripheral formation of alanine. The augmented synthesis of alanine is a consequence of insulin's action on glucose translocation, which leads to greater availability of glucose-derived pyruvate for transamination.[42,51] The tendency for insulin to increase muscle uptake of circulating alanine is thus counterbalanced by augmented intracellular production and release of this amino acid. Consequently, the net effect of insulin on circulating levels of alanine is either a failure to induce a consistent reduction, or in some cases, an increase may occur. In a like manner the availability of glucose or pyruvate to the insulin-treated rat diaphragm results in enhanced alanine formation by the incubated tissues and dilution of the labeled alanine in a greater intracellular pool of unlabeled amino acid.[51,52] These unique aspects of the interaction of insulin and alanine metabolism thus support the notion that alanine is synthesized peripherally and that its carbon skeleton represents an important end product of peripheral glucose utilization.

Since insulin does not decrease net availability of circulating alanine, the manner whereby insulin reduces hepatic gluconeogenesis must depend on a hepatic rather than a peripheral effect. Evidence to support this conclusion has recently been provided by Felig and Wahren in studies in intact man[55] (Fig. 4). Glucose was administered intravenously to normal subjects in a dose of 25 mg/kg/min for 20 min, causing a fivefold or more increment in peripheral insulin levels and a reversal in hepatic glucose balance from a net output to a

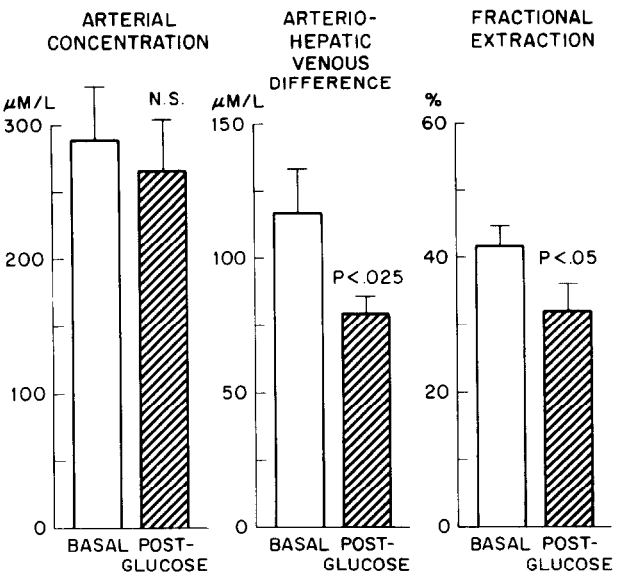

Fig. 4. Arterial concentration and splanchnic extraction of plasma alanine in basal state and after stimulation of endogenous insulin by infusion of glucose (25 mg/kg/min). Glucose infusion failed to significantly reduce arterial alanine levels. Nevertheless, net spanchnic uptake of alanine fell, as a consequence of a reduction in fractional extraction. (By permission.[55])

net uptake. In association with the hyperinsulinemia and inhibition of hepatic glucose output, splanchnic uptake of alanine and other glycogenic amino acids fell by 30%–60%. This reduction in alanine uptake was entirely a consequence of a decrease in splanchnic fractional extraction of this amino acid. In marked contrast, and as predicted from the studies cited above, the glucose infusion failed to reduce significantly the levels of circulating alanine.[55] Further evidence of a direct hepatic effect of insulin in regulating gluconeogenesis from alanine has been provided from studies in perfused liver in which insulin has been observed to inhibit incorporation of ^{14}C alanine into glucose.[56]

At first glance, these inhibitory effects of insulin on hepatic uptake and utilization of alanine would seem to be at variance with the widely held notion that insulin stimulates cellular uptake of amino acids. However, it should be emphasized that whereas a stimulatory effect of insulin on uptake and incorporation of amino acids into protein is readily demonstrable in muscle (as reviewed above) and in fat cells,[57] such is not the case with respect to the normal liver. Thus, in perfused livers from normal rats, insulin is without effect or at best has an equivocal stimulatory action on amino acid incorporation into liver protein.[58,59] Similarly, in intact animals the insulin-mediated nitrogen sparing action of glucose is associated with increased synthesis of protein in muscle but not in liver.[60] These observations are thus in keeping with the notion that the flux of amino acids from muscle to liver in post-absorptive man, in which alanine predominates, is not directed at promoting the synthesis of liver proteins but serves to provide substrate for gluconeogenesis.

ALANINE METABOLISM IN DIABETES

An increase in total plasma amino acid content was reported in pancreatectomized dogs[61] and in diabetic patients,[62] even before the hypoaminoacid-

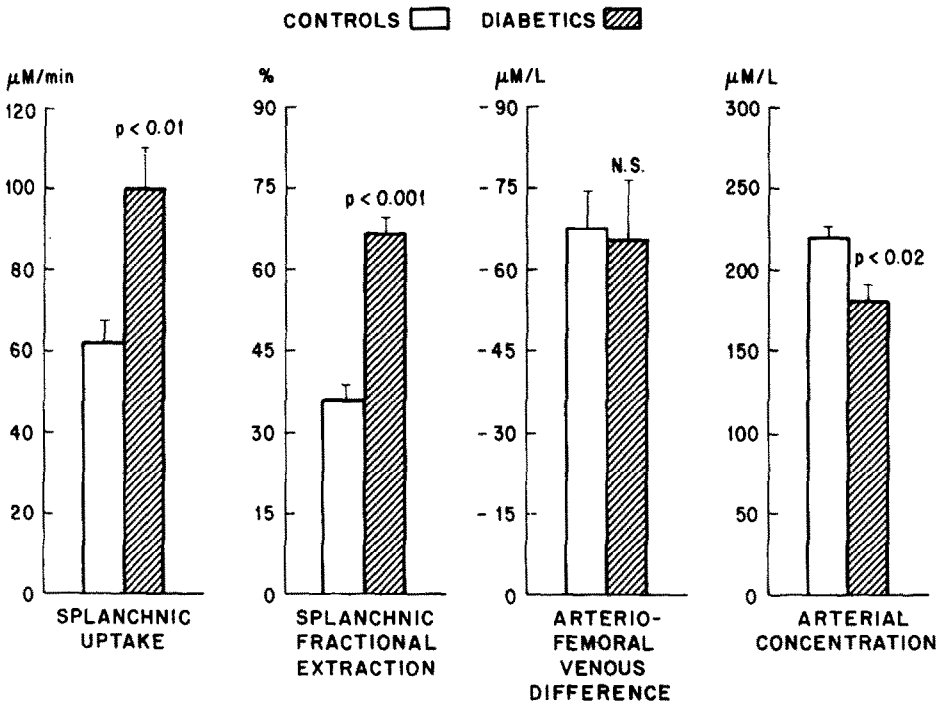

Fig. 5. Arterial concentration, splanchnic exchange and arterio-femoral venous differences for plasma alanine in insulin-dependent diabetics and normal subjects. Despite a reduction in arterial alanine concentration, splanchnic uptake is increased in the diabetics as a consequence of augmented fractional extraction.

emic effect of insulin was discovered.[36] However, as in the case of insulin administration, the significance of these findings with regard to glucoregulatory mechanisms can be determined only in light of the changes in the pattern of individual amino acids. Examination of individual amino acid levels reveals that the increment in amino acid content in diabetes is due almost entirely to an accumulation of the branched-chain amino acids.[42,63,64,65,66,67] In marked contrast, alanine levels repeatedly have been observed to be reduced in diabetes.[42,64,65,66,67] Administration of insulin tends to restore plasma alanine levels toward normal.[42] The diminution in alanine concentration observed in diabetic ketoacidosis, a condition characterized by gross overproduction of glucose,[68] suggested to Felig et al. that augmented gluconeogenesis in diabetes is due to a primary increase in hepatic amino acid extraction rather than a consequence of augmented precursor availability.[64] More direct evidence to support this conclusion has been provided by recent studies of splanchnic amino acid exchange in insulin-dependent diabetics.[67] Although the patients investigated were hyperglycemic (blood glucose 250–350 mg/100 ml), residual insulin activity was available at the time of the study to maintain total glucose production in the range comparable to that observed in nondiabetic subjects. Nevertheless, splanchnic uptake of alanine and other glucose precursors was

increased 60%–100%, so that the relative contribution of gluconeogenesis to total glucose output was increased by more than 50% in the diabetics. Particularly noteworthy was the observation that augmented alanine uptake was entirely a consequence of a doubling of the splanchnic fractional extraction of this amino acid, whereas the arterial concentration of alanine was reduced by 20% (Fig. 5). These data thus indicate that augmented gluconeogenesis from alanine in diabetes is a consequence of a primary alteration in hepatic processes rather than a secondary result of an increased supply of gluconeogenic precursors.[67] It should be noted that direct evidence of increased conversion of alanine to glucose was not obtained in the aforementioned investigation in diabetic patients. A decrease in the intracellular content of alanine has been observed, however, in the livers of alloxan-diabetic rats.[69] The demonstration that hepatic uptake of alanine is augmented in diabetes, coupled with evidence of decreased intracellular accumulation of this amino acid in a setting of overall negative nitrogen balance, suggests a primary stimulation of disposal of alanine along gluconeogenic pathways.

ALANINE–GLUCAGON RELATIONSHIPS

In addition to its well-recognized glycogenolytic effects, glucagon is believed to contribute to glucose homeostasis by stimulating gluconeogenesis.[70] Increased incorporation of ^{14}C-alanine into glucose in the presence of glucagon has been demonstrated with the isolated perfused rat liver.[4,71] Although the uptake by the perfused liver of a number of glycogenic amino acids is stimulated by glucagon, the magnitude of this increase is greatest for alanine.[72] Changes in the steady state concentrations of intermediates in the gluconeogenic pathway observed in the perfused liver suggest that glucagon acts to enhance gluconeogenesis from alanine by stimulating the conversion of pyruvate to phosphoenolpyruvate and by increasing the transport of this amino acid into the hepatic cell.[4] However, inasmuch as both the extra- and intracellular concentrations of alanine decrease in the glucagon-treated perfused liver, intracellular utilization of this amino acid (presumably for conversion to glucose) is apparently stimulated to a greater extent than inward transport.[72]

The in vivo evidence for a gluconeogenic effect of glucagon in intact man and experimental animals is less compelling than that observed with the perfused liver. Splanchnic balance data suggest that the acute increase in hepatic glucose production induced by glucagon in normal postabsorptive man is primarily a consequence of enhanced glycogenolysis.[63] Nevertheless, a protein catabolic effect of glucagon (as evidenced by negative nitrogen balance) has been demonstrated in fed,[74] fasted,[75] and diabetic subjects.[76] In addition, glucagon administration results in a two- to fourfold increase in splanchnic uptake of total alpha amino nitrogen[73,77] in the face of a reduction in total plasma amino acid concentration.[77] Furthermore, following a 3-day fast, at which time liver glycogen stores are depleted,[78] alanine administration results in an increase in blood glucose that is proportional to the rise in plasma glucagon.[79] Thus, while the acute hyperglycemic effect of glucagon

in postabsorptive subjects is predominantly a consequence of glycogenolysis, gluconeogenic processes are apparently stimulated as well.

As in the case of the perfused liver, examination of the effect of glucagon on individual plasma amino acids in intact man substantiates the important role of alanine as a gluconeogenic precursor. Thus, in both the fasted[80,81] and postprandial state,[80] the magnitude of the decline in plasma alanine induced by glucagon exceeds that of virtually all other amino acids. That the hypoalaninemic effect of glucagon is largely a reflection of hepatic utilization and conversion to glucose is suggested by the failure to observe such a decline in patients with acute viral hepatitis and impaired glucose homeostasis.[81] It is of interest that in contrast to the key endogenous glycogenic amino acids, the decrease in the plasma concentration of the branched-chain amino acids induced by large doses of glucagon is insulin-mediated and extrasplanchnic in origin.[82] On the other hand, the possibility that glucagon may also influence the extrahepatic metabolism of alanine and other glycogenic amino acids is suggested by the demonstration that infusion of small doses of glucagon (0.1 mg/24 hr) in obese subjects fasted for 5–6 wk results in hypoaminoacidemia, yet fails to increase urea excretion.[75]

The interaction between glucagon and alanine extends beyond that of hormone and target substrate. Müller et al. demonstrated that infusion of alanine in dogs results in a consistent increase in plasma glucagon concentration.[83] The stimulatory effect of alanine on glucagon secretion was inhibited when gluconeogenic requirements were obliterated by infusion of glucose.[83] Recent studies have extended these observations to intact man in whom the alpha cell response to alanine is augmented by fasting and diabetes,[79] diminished by obesity,[84] and reflected in a direct linear relationship by the increase in blood glucose.[79] Whether physiologic increments in plasma alanine (as observed in exercise and postprandially) can increase plasma glucagon secretion in man has not been established. Nevertheless, the data available suggest that glucagon may play a pivotal role in the cyclical interrelationship between alanine and glucose.[83]

CORTICOSTEROIDS AND ALANINE METABOLISM

An important role of corticosteroids in the regulation of gluconeogenesis has been apparent since the classic studies of Long et al. indicated that catabolism of body protein provides the precursors for steroid-induced production of carbohydrate.[85] Despite innumerable studies over the subsequent 30 yr demonstrating a variety of enzymatic and metabolic alterations following glucocorticoid administration, the mechanism whereby these hormones influence gluconeogenesis has not been firmly established.[86] Specifically, glucocorticoids may act primarily to augment precursor supply by influencing peripheral amino acid release,[87] or alternatively they may have a direct stimulatory action on hepatic enzymatic mechanisms.[88] With regard to the effects of steroids on alanine metabolism, the data are somewhat confusing. However, many of the seeming discrepancies can be explained on the basis of differences in experimental design involving varying concentrations of substrate and differences in duration of hormonal treatment.

In general, an in vitro effect of glucocorticoids on gluconeogenesis has been difficult to demonstrate with the perfused rat liver preparation.[86] Nevertheless, Eisenstein et al. noted that glucose formation from alanine is impaired in livers obtained from adrenalectomized rats and is restored to normal by addition of a potent glucocorticoid to the perfusion medium.[89] It should be noted, however, that the concentration of alanine employed by Eisenstein et al. was 10 m*M* or 20–30 times the circulating physiologic level of this amino acid. In studies involving physiologic concentrations of alanine, Haft et al. observed normal rates of incorporation of this amino acid into glucose by livers obtained from adrenalectomized rats and concluded that hepatic gluconeogenesis is normal in adrenal insufficiency if precursor supply is adequate.[90] It would thus appear that the effect of steroids on the liver is to increase the maximal capacity for hepatic gluconeogenesis in circumstances of marked precursor overload.

With regard to the effect of steroids on peripheral alanine metabolism, it has been well documented that acute administration of steroids accelerates the accumulation of total free amino acids in the plasma of eviscerated animals suggesting an enhanced output from peripheral protein stores.[80] Direct evidence that adrenal steroids stimulate amino acid release from muscle has been provided by in vitro studies with the isolated rat diaphragm.[91] However, more recent observations in intact man in whom individual amino acids have been measured have been less supportive of a primary stimulatory effect of glucocorticoids on peripheral amino acid release. Thus, Soupurt observed a decrease in the concentration of plasma amino acids in patients treated for prolonged periods with hydrocortisone.[92] On the other hand, Zinneman et al. noted that cortisol administration for 3–5 days failed to alter plasma amino acid levels.[93] The seeming discrepancy between these data in intact man and prior studies in animals is resolved, however, by the data of Ryan and Carver.[94] These authors observed that 24 hr after the injection of hydrocortisone, the concentration of free amino acids in the plasma and muscle is increased in the rat. In contrast, 10 days of repeated treatment with hydrocortisone results in a diminution in plasma amino acids. These data thus indicate that glucocorticoids initially augment plasma amino acid levels but this action is dissipated with chronic administration.

It is noteworthy that the absolue magnitude of the acute increment in plasma concentration of individual amino acids reported by Ryan and Carver was greatest for alanine.[94] Similarly, Betheil et al. noted that the increment in plasma and liver concentration of alanine induced by cortisone was among the highest observed for any amino acid.[95] Furthermore, Pagliara et al.[96] have recently reported a doubling of plasma alanine levels 4 hr after administration of 40 mg of cortisone acetate to a patient with ketotic hypoglycemia (see below). Although direct studies of muscle alanine release are not available in adrenal insufficiency, diminished incorporation of ^{14}C alanine into blood glucose in adrenalectomized rats in the face of normal rates of gluconeogenesis from lactate is compatible with a decreased output of this precursor from peripheral protein stores in the absence of corticosteroids.[97]

In summary, glucocorticoids acutely stimulate the release of amino acids

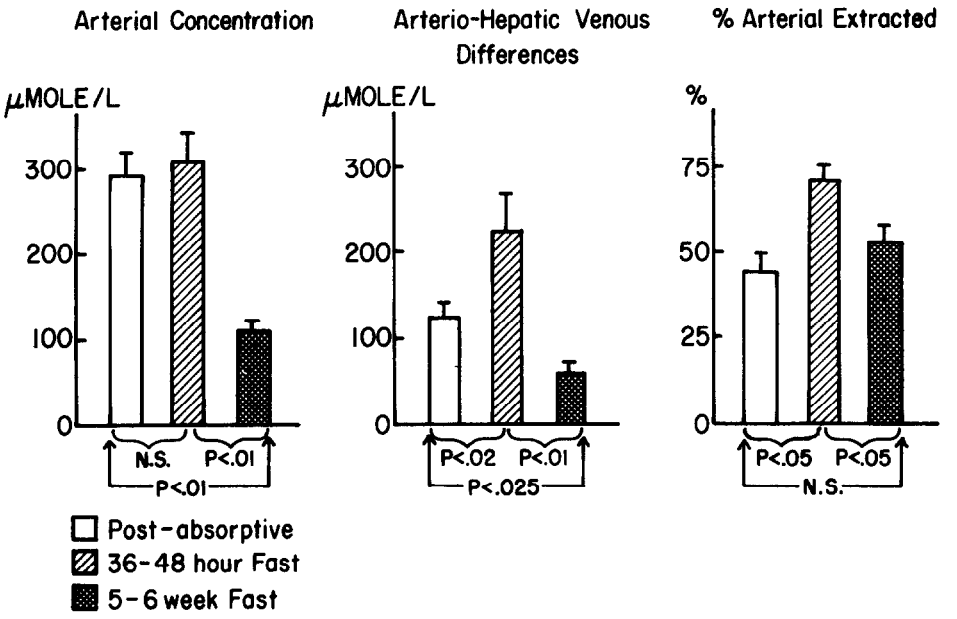

Fig. 6. Alanine metabolism during brief and prolonged starvation. After a brief fast extraction of alanine is increased. In contrast, following a prolonged fast, splanchnic alanine uptake is reduced, solely as a consequence of decreased arterial levels inasmuch as fractional extraction of this amino acid is not significantly different from the postabsorptive state. (By permission.[2])

from muscle resulting in their accumulation in plasma and greater uptake by the liver. Both with regard to the increase in plasma concentration as well as availability within the liver cell, the effect of glucocorticoids on alanine exceeds that of most other amino acids. Corticosteroids also act directly on the liver to increase the maximal capacity for gluconeogenesis from alanine, but this effect is demonstrable only with very high and generally unphysiologic substrate concentrations.

ALANINE AND GLUCONEOGENESIS IN PROLONGED STARVATION

The key role of alanine in the regulation of gluconeogenesis is particularly evident when one examines carbohydrate and protein metabolism in prolonged fasting. It has been recognized since the classic studies of Benedict that prolonged starvation is characterized by a progressive decline in the rate of protein catabolism.[98] This is manifested by a reduction in urinary nitrogen loss from levels of 12–15 g/day during the first wk of fasting to less than 5 g/day after 5–6 wk of starvation.[99] Since protein represents the sole source of de novo glucose formation in mammalian tissue,[100] such a reduction in protein breakdown must be accompanied by a concomitant decline in gluconeogenesis. Indeed, studies employing the hepatic and renal venous catheter technique have demonstrated that total glucose production falls to less than 90 g/day after a 5–6 wk fast with the liver contributing no more than 55%. That hormonal changes are not the prime regulatory factors in this response is suggested by

the persistence of hypoinsulinemia[99] and hyperglucagonemia[75] throughout starvation. Such a milieu would favor augmented rather than diminished gluconeogenesis. In contrast, changes in alanine metabolism provide some insight as to the mechanism underlying the decline in glucose production.

In prolonged fasting, the plasma concentrations of most aminoacids ultimately decline.[2] However, the magnitude of this diminution in both absolute and relative terms, and the rapidity with which it occurs is most marked in the case of alanine.[2,101] Thus, after a 5–6-wk fast alanine concentration has fallen by 70%. Accompanying this lessened availability of circulating alanine is a corresponding fall in splanchnic uptake of this amino acid.[2] On the other hand, splanchnic fractional extraction of alanine remains unaltered from the postabsorptive state (Fig. 6). These changes in alanine metabolism thus indicate that decreased substrate availability (as evidenced by hypoalaninemia) rather than primary inhibition of hepatic processes appears to be the rate limiting factor in the regulation of hepatic gluconeogenesis in prolonged fasting.[2] Supporting this conclusion is the observation that despite the overall reduction in hepatic glucose output, incorporation of ^{14}C-alanine into blood glucose is no less rapid in prolonged starvation than in the overnight fasted condition.[5,29] Evidence of lessened availability to the liver of this key glycogenic substrate in starvation is also indicated by studies in experimental animals. In the livers of fasted rats, the intracellular content of free alanine falls to a greater extent than that of all other amino acids.[69]

The mechanism whereby hypoalaninemia is maintained in starvation is indicated by examination of amino acid exchange across the deep venous bed of the forearm. Although alanine remains the major amino acid released by muscle tissue after prolonged fasting, its rate of output declines by over 70% after a 5–6-wk fast.[6] Some of this decline may be related to decreased glucose utilization and lessened pyruvate availability. However, since the output of virtually all amino acids falls,[6] it is likely that diminished proteolysis is the primary controlling factor.

Studies in which exogenous alanine has been administered to fasted subjects provide further evidence of the importance of availability of this amino acid in the control of gluconeogenesis in starvation. Thus, intravenous infusion of alanine after a 4–6-wk fast results in a prompt increase in blood glucose concentration.[3] A similar glycemic effect is not observed, however, after infusion of glycine.[3] Furthermore, oral intake of alanine in a calorically trivial dose of 50 g/day restores blood glucose to prefast levels and diminishes urinary ammonia excretion.[10]

The question may be raised as to whether the changes in plasma alanine observed in prolonged starvation are a consequence of inadequate caloric intake per se (irrespective of the dietary source of calories), lack of carbohydrate, or a result of protein depletion. As to the last possibility, isocaloric replacement of protein by carbohydrate (i.e. ingestion of a high carbohydrate, protein-free diet), results in a marked elevation rather than a reduction in plasma alanine levels.[101,103,104,105] In contrast, isocaloric, isonitrogenous replacement of carbohydrate by fat results in a specific fall in plasma alanine

concentration.[106] These dietary studies thus indicate that plasma alanine levels are reduced in circumstances in which the need for gluconeogenesis is augmented (starvation, carbohydrate lack). On the other hand, when an excess of dietary carbohydrate is available, even in the face of protein lack, alanine accumulates in blood, presumably as a consequence of decreased consumption for gluconeogenesis.

ALANINE DEFICIENCY STATES

Of prime relevance to our understanding of the glucose–alanine cycle has been the recent demonstration by a number of laboratories that hypoalaninemia is a concomitant of a variety of conditions characterized by fasting hypoglycemia and/or deficient gluconeogenesis. Specifically, decreased availability of alanine has been implicated in the alterations in glucose homeostasis observed in pregnancy,[107] ketotic hypoglycemia of infancy,[96] and following ethanol ingestion.[30]

Studies in humans[108] as well as experimental animals[109] have demonstrated that the hypoglycemic, hyperketonemic, and hypoinsulinemic response to starvation is accelerated and exaggerated in normal pregnancy. Continuous glucose consumption by the fetal-placental unit appears to be the factor initiating this sequence of events.[108,110] However, the failure of maternal gluconeogenic mechanisms to keep pace with the total glucose demands of maternal and fetal tissues as reflected by the development of hypoglycemia indicates that factors are operative in pregnancy which serve to limit maternal glucose production. That direct inhibition of intrahepatic processes is not responsible for this limitation is indicated by the augmented rather than diminished capacity of the liver to convert exogenous precursors to glucose.[111] On the other hand, studies of plasma amino acid levels suggest that substrate lack contributes to the development of gestational hypoglycemia.[107,112] Thus, in pregnant women plasma alanine levels are reduced after an overnight fast and fall more rapidly than in nonpregnant controls as fasting progresses[107] (Fig. 7). On the other hand, when substrate is made available in the form of exogenous alanine, a comparable glycemic response is observed in pregnant and nonpregnant women.[107] Whether increased placental uptake, diminished maternal release, or altered placental hormone secretion[113] is responsible for the diminished availability of alanine in pregnancy remains to be determined.

Ketotic hypoglycemia, the most common form of hypoglycemia encountered in young children, is a disorder characterized by recurrent episodes of hypoglycemia and ketosis that can be provoked by fasting or ingestion of a low carbohydrate, high fat intake.[114,115] Neither hyperinsulinemia nor abnormalities in the metabolism of such glucose precursors as fructose or glycerol are present in this disorder.[114,115,116] Evidence of a deficiency of alanine in ketotic hypoglycemia has recently been reported by Pagliara et al.[96] These authors demonstrated that alanine levels in children with this syndrome were reduced by 30% after an overnight fast and fell to lower levels than observed in normal children during ingestion of a provocative hypocaloric, low-carbohydrate diet. Infusion of exogenous alanine or administration of cortisone acetate, which

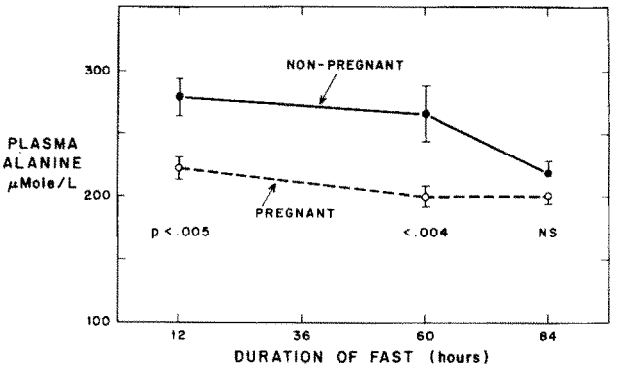

Fig. 7. Plasma alanine levels in pregnant and nonpregnant subjects during an 84-hr fast. Hypoalaninemia contributes to fasting hypoglycemia in pregnancy. (By permission.[107])

raised endogenous alanine levels, promptly restored blood glucose levels to normal. These data thus suggest that a deficiency in alanine rather than a defect in intrahepatic processes is the primary pathogenetic factor in ketotic hypoglycemia.[96]

The hypoglycemic effects of ethanol (in appropriately fasted individuals), and its interference with gluconeogenesis from lactate have been well established.[117,118] Recently, Kreisberg et al. reported that ethanol also inhibits gluconeogenesis from alanine.[30] Of particular interest was their observation that in contrast to lactate, the plasma concentration of alanine was significantly reduced after ethanol administration. The possibility was suggested that acetate and/or lactate arising from ethanol metabolism may interfere with the synthesis and release of alanine from muscle by inhibiting peripheral glucose utilization and thereby limiting pyruvate availability.[30] Irrespective of the mechanism of hypoalaninemia, substrate deficiency appears to be a contributory factor to ethanol-mediated inhibition of gluconeogenesis.

HYPERALANINEMIC CONDITIONS

The interaction between alanine formation and glucose metabolism has been further substantiated by the identification of a variety of conditions in which augmented glucose utilization and/or accumulation of glycolytic intermediates is accompanied by an elevation in plasma alanine concentration. Hyperalaninemia has been noted in physiologic circumstances such as exercise,[12,119] in inborn errors of metabolism characterized by chronic hyperpyruvicemia,[17,18] and in acquired acute lactic acidosis.[120]

Amino acid exchange across the leg and splanchnic bed was examined in subjects exercising on a bicycle ergometer at mild (400 kg-m/min), moderate (800 kg-m/min) and severe (1200 kg-m/min) work loads.[12] At all levels of work intensity net amino acid release across the exercising leg was observed only for alanine. (In this respect exercising skeletal muscle is comparable to cardiac muscle, which also releases ony alanine.[13]) Furthermore, leg alanine output increased above resting levels in proportion to the work load (Fig. 8). Arterial alanine concentration was directly proportional to arterial pyruvate levels both at rest and during exercise, and rose by 25%–100% with exercise. In contrast, the concentrations of all other amino acids remained unchanged

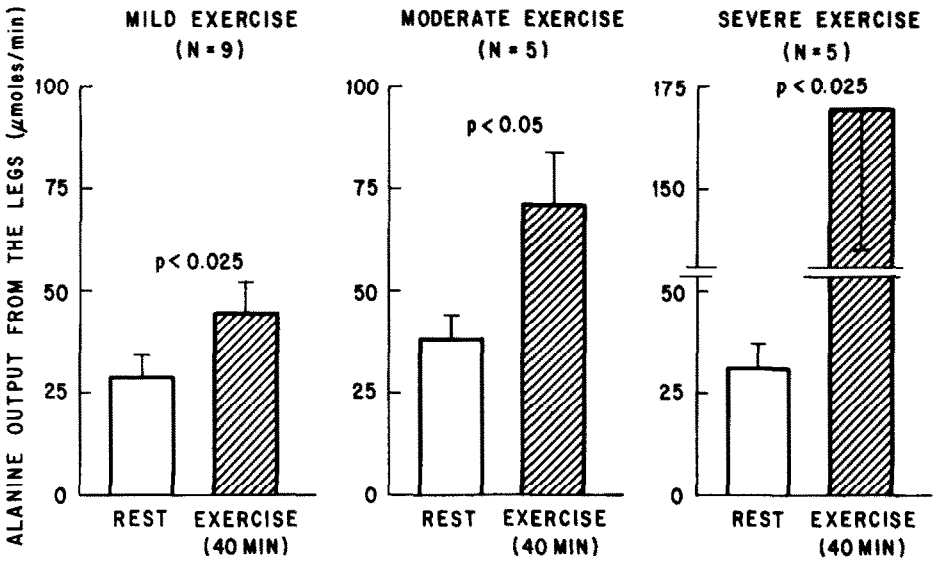

Fig. 8. Influence of exercise on alanine output from the legs. Increase in alanine output was proportional to intensity of the exercise. (By permission.[12])

during mild exercise, while at heavy loads, small increments were noted, which were attributable to altered splanchnic exchange rather than augmented peripheral release.[12]

These observations in exercising man thus underscore the special role of alanine in amino acid metabolism. The data suggest that synthesis of alanine is increased in exercise, as a consequence of increased availability of glucose-derived pyruvate and amino groups.[12] As to the source of the latter, it has long been recognized that exercise is accompanied by increased peripheral release of ammonia,[121,122,123] indicating augmented breakdown of amino acids. Besides the branched-chain amino acids, which are preferentially catabolized in muscle,[19,20] there is evidence of enhanced transfer of amino groups from aspartate in exercise. Thus, increased formation of oxaloacetate from aspartate has been reported in association with augmented activity of the tricarboxylic acid cycle.[124,125] In addition, exercise results in a cyclic interconversion of purine nucleotides, which is accompanied by conversion of aspartate to fumarate and liberation of ammonia.[126]

With regard to the origin of the pyruvate for peripheral alanine synthesis, the increase in glucose consumption by the exercising leg[127] and the rise in arterial pyruvate levels,[12] suggest augmented peripheral availability of this glycolytic intermediate for transamination to alanine. By this formulation, the carbon skeleton of alanine represents an important end product of glycolysis in exercising man and the rate of alanine formation is not solely dependent on protein catabolism but on pyruvate formation as well. A unique opportunity to test this hypothesis was recently provided by a patient with McArdle's syndrome.[128] In this disorder, myophosphorylase is lacking and as a

consequence muscle glycogen cannot be utilized to meet the energy requirements of muscle contraction.[129] In contrast to normal subjects, in McArdle's syndrome exercise results in a fall in arterial pyruvate and lactate levels,[129] and in a decrease in muscle pyruvate content.[128] It is thus noteworthy that in association with this exercise-induced diminution in pyruvate levels in McArdle's syndrome, a progressive decline rather than an increment was also observed in plasma alanine concentration.[128] Furthermore, a significant net uptake of alanine rather than an output was demonstrated across the exercising leg.[128] Thus, in circumstances in which pyruvate formation is limited, exercise of itself fails to augment peripheral alanine production.

It should be noted that at all levels of exercise in normal subjects, splanchnic uptake of alanine exceeds that of all other amino acids.[12] Although splanchnic blood flow is markedly reduced in exercise, the rate of splanchnic alanine uptake remains comparable to resting levels because fractional extraction of this amino acid is increased.[12] The overall influence of exercise on the glucose-alanine cycle is thus to stimulate peripheral alanine formation while hepatic consumption of this amino acid remains essentially unchanged. As a consequence, alanine accumulates in arterial blood. The specificity of exercise-induced hyperalaninemia has recently been confirmed in experimental animals by Christophe et al., who noted small reductions in the levels of seven plasma amino acids in rats after 15–30 min of forced swimming; in contrast, plasma alanine was unique in demonstrating an increase.[130]

In the last several years, an increase in plasma alanine in the absence of generalized hyperaminoacidemia has been observed in a number of children with chronic hyperpyruvicemia.[17,18,131,132] Lonsdale et al. reported a 5-yr-old boy with optic atrophy and intermittent ataxia, in whom the ataxic episodes were characterized by elevations in plasma and urinary pyruvate, lactate, and alanine.[17] Administration of pharmacologic doses of vitamin B complex resulted in a reduction in the levels of these metabolites. Lonsdale et al. suggested that the condition in their patient represented a thiamine dependency state, in which oxidative decarboxylation of pyruvate to acetate was blocked.[17] In a patient with a similar movement disorder associated with hyperpyruvicemia and hyperalaninemia, Blass et al. demonstrated a marked decrease in pyruvate decarboxylase activity in cultured skin fibroblasts and white blood cells.[132] Hyperalaninemia with pyruvicemia was also reported by Yoshida et al. in a 10-yr-old girl with mental retardation.[18] Biochemical examination of a liver biopsy specimen revealed decreased activity of pyruvate carboxylase and decreased incorporation of pyruvate into glycogen. The site of the biochemical lesion in this patient was thus believed to involve the conversion of pyruvate to oxaloacetate, a key step in the gluconeogenic pathway.[18] Interestingly, hyperalaninemia in association with pyruvate and lactate accumulation has also been reported in two microcephalic sisters with diabetes mellitus.[131] Glucose infusion resulted in an excessive rise in plasma pyruvate, lactate, and alanine.[131] These data as well as the presence of fasting hyperglycemia rather than hypoglycemia, suggest that blockade of pyruvate oxidation rather than inhibition of its utilization for gluconeogenesis (carboxylation to oxaloacetate) is the

underlying mechanism in at least some cases of chronic hyperpyruvicemia. Whatever the basis of pyruvate accumulation, it is noteworthy that alanine levels are increased in each of these children, in the absence of elevations in other plasma amino acids. Similarly, elevated alanine levels were observed in the single case of chronic lactic and pyruvic acidosis reported thus far in an adult.[133]

Marked elevations in plasma alanine have recently been reported in the acute form of severe lactic acidosis observed in adults.[120] Although the levels of 15 of 19 amino acids were above the normal range, the increment was greatest for alanine (1–5 m*M*/liter), and was proportionate to the rise in pyruvate.[120] Since high concentrations of lactate and pyruvate were observed to inhibit alanine utilization in the perfused rat liver, it was suggested that hepatic disposal of alanine is impaired in lactic acidosis.[120] However, since several of the patients manifested circulatory collapse, this impairment in alanine utilization need not reflect a direct hepatotoxic effect of lactate and pyruvate, but could be equally well explained by a reduction in splanchnic perfusion.

QUANTITATIVE CONSIDERATIONS

In the preceding discussion the special role of alanine has been emphasized by describing the many aspects and situations in which its metabolism differs from that of other amino acids and is intimately related to glucose homeostasis. While the relative importance of alanine is thus readily apparent, full evaluation of the significance of the glucose–alanine cycle necessitates an examination of its quantitative contribution to overall glucose balance. Two specific questions warrant consideration. (1) What proportion of hepatic glucose output can be accounted for by gluconeogenesis from alanine? (2) What fraction of muscle glucose uptake can be accounted for by peripheral production of alanine? These questions can best be answered by examining the overall production and utilization rates of glucose and its precursor substrates (Fig. 9).

The rate of glucose production has been estimated in normal man by the hepatic venous catheter technique as well as by isotope dilution methods employing ^{14}C-glucose. Since a variety of units have been employed by various authors in expressing glucose turnover rates (mg/kg/min, mg/kg/hr, mmole/min, mg/sq m/min), for the purpose of this discussion, the data from these studies will be converted to g/24 hr. In this calculation, the observed rate of glucose production is extrapolated to a 24-hr period.

In the postabsorptive state, the liver is essentially the sole source of glucose production, extrahepatic glucose production being negligible.[100] Furthermore, the glucose uptake by the tissues drained by the portal vein is of such small magnitude[134] that splanchnic glucose production underestimates the true hepatic glucose output by less than 5%.[127] Under normal circumstances, in the postabsorptive state (8–14-hr fast), glucose release by the splanchnic bed, as determined by arterio-hepatic venous differences, occurs at a rate of 160–350 g/day. (The specific values reported are 160,[134] 208,[127] 250,[67] 280,[135] 340,[55] and 350 g/day.[136]) Estimates of glucose turnover as determined by

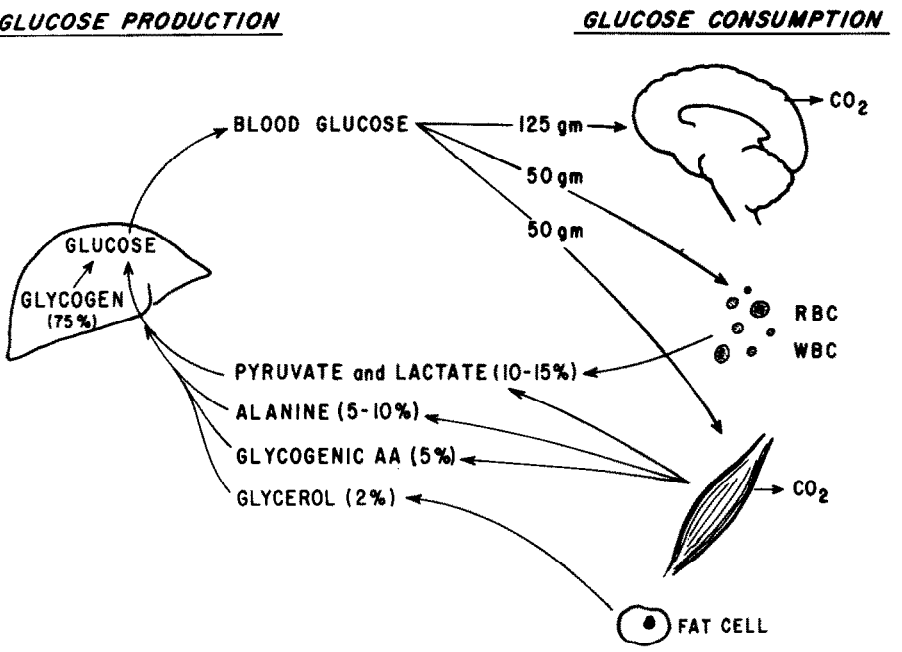

Fig. 9. Balance of glucose and glucose precursors in postabsorptive man. The brain is the prime site of glucose consumption. Most of the glucose released by the liver is derived by glycogenolysis. As fasting progresses, gluconeogenesis replaces glycogenolysis as the predominant mode of glucose formation. Numbers in parentheses represent proportion of glucose output attributable to uptake of various precursors in postabsorptive state.

isotope techniques are somewhat lower, ranging from 200[31,137,138,139] to 250 g/day.[140,141]

The glucose released by the liver is derived by breakdown of liver glycogen and by gluconeogenesis from lactate, pyruvate, glycerol, and amino acids. With regard to gluconeogenesis, the proportion of total glucose production, which can be accounted for by uptake and utilization of lactate, as determined by splanchnic balance studies ranges from 10%–15%.[26,55,127] These values are in good agreement with the recycling rate of 10%–16% reported by isotope methods.[31,118,139,140,142] The levels of circulating pyruvate and glycerol in postabsorptive man are so much lower than that of lactate, that despite virtually complete extraction of the former by the liver, their relative contributions are no greater than 1% and 2%, respectively, to total glucose output.[127,143] The proportion of glucose production accounted for by uptake of alanine as determined by splanchnic balance studies, ranges from 5%–12%.[2,26,55,127] Since in contrast to the other precursors, alanine is released to some extent by the GI tract,[12] the splanchnic balance data represent a small underestimate of true hepatic utilization of the substrate. The remaining amino acids contribute an additional 5% to the total glucose production.[2,55]

From the above data, it is apparent that total precursor uptake accounts for no more than 20%–30% of hepatic glucose production in the postabsorp-

tive state (Fig. 9). The remaining 70%–80% not accounted for by gluconeogenesis from circulating precursors is presumably derived by breakdown of liver glycogen. Direct evidence indicating that hepatic glycogen is the primary source of glucose in the postabsorptive state has recently been provided by Hultman and Nilsson.[78] These authors demonstrated that after an overnight fast, glycogen is present in the liver in a concentration of 50 g/kg of liver tissue, representing a total of 80–90 g of glucose. Repeated liver biopsies over the ensuing 4 hr indicated that glycogen is broken down at such a rate so as to release 90–100 mg of glucose/min.[78] This glycogenolytic rate is considerably greater than the gluconeogenic rate of 40–50 mg of glucose/min, and is sufficient to account for the proportion of glucose output not attributable to precursor uptake.

It should be noted that while alanine uptake accounts for no more than 12% of hepatic glucose output in the postabsorptive state, it nevertheless is responsible for 25%–30% of the total contribution from gluconeogenesis. The relative significance of the glucose–alanine cycle in total glucose production will thus depend on the extent to which gluconeogenic as opposed to glycogenolytic processes predominate. Accordingly, as fasting extends for periods of 24 hr or more and glycogen stores are totally dissipidated,[78] gluconeogenesis rather than glycogenolysis becomes the dominant mode of hepatic glucose production. Thus, after a 48–72-hr fast, alanine uptake accounts for 26% of hepatic glucose output.[2] Consequently, a deficiency of alanine as seen in pregnancy[107] and in ketotic hypoglycemia of infancy,[96] results in an exaggerated fall in blood glucose after relatively brief periods of fasting.

With regard to glucose utilization, the estimated rates of cerebral glucose uptake of 125–150 g/day,[144,145] indicate that the bulk of the glucose produced under basal, postabsorptive circumstances is terminally oxidized by the brain. Smaller amounts of glucose are taken up by obligate glycolytic tissues such as the formed elements of the blood, and by muscle.[146] Direct determinations

Table 1. Proportion of Leg and Forearm Muscle Glucose Uptake Accounted for by Alanine Production (A/G) in the Resting State[6,12,127]

Leg	
A-FV Glucose*	= 184 μmole/liter
A-FV Alanine	= −68 μmole/liter
A-FV Lysine	= −19 μmole/liter
A-FV "Glucose-derived Alanine"†	= −49 μmole/liter
A/G‡	= 13%
Forearm muscle	
A-DV Glucose§	= 211 μmole/liter
A-DV Alanine	= −111 μmole/liter
A-DV Lysine	= −37 μmole/liter
A-DV "Glucose-derived Alanine"†	= −74 μmole/liter
A/G‡	= 18%

*A-FV: Arterio-femoral venous difference.
†A-V "Glucose-derived Alanine": (A-V) Alanine — (A-V) Lysine.
‡A/G: 100 (½ "glucose-derived alanine" V-A difference)/(Glucose A=V difference).
§ A-DV: Arterio-deep venous difference.

of the proportion of muscle glucose uptake which ultimately appears as the carbon skeleton of alanine have not been reported. However, from the available balance data, gross estimates may be made (Table 1). Since lysine does not undergo transamination, peripheral release serves as an index of proteolysis. In addition, the concentration of alanine in muscle is quite similar to that of lysine.[15] Accordingly, the extent to which the output of alanine exceeds that of lysine may reflect the proportion of alanine synthesized by transamination of glucose-derived pyruvate. By this formulation 13% and 18% of glucose uptake by the leg and deep tissues of the forearm respectively, may be accounted for by alanine production (Table 1). It is of interest that 20%–40% of glucose uptake by resting forearm muscle is estimated to be disposed of as lactate.[147,148] Thus in terms of its contribution to peripheral glucose utilization as well as its role in hepatic glucose formation, recycling of carbon skeletons along the glucose-alanine pathway in resting postabsorptive man appears to occur at a rate that is approximately 50% of that which is observed for the lactate (Cori) cycle.

ALANINE AND NITROGEN METABOLISM

Although this discussion has focused on the relation of alanine to glucose homeostasis, it is obvious that the glucose-alanine cycle is of importance in nitrogen metabolism as well. In addition to providing carbon skeletons for gluconeogenesis, the net effect of peripheral synthesis of alanine and its subsequent uptake by the liver is the transfer of amino groups from muscle to hepatic tissues, where they may be disposed of as urea. It has been suggested, therefore, that alanine provides a nontoxic alternative to ammonia in the transport of nitrogen from peripheral tissues to the liver.[26] Supporting this carrier role of alanine is the observation that the plasma concentration of this amino acid is increased in a variety of hyperammonemic situations. Thus, in familial protein intolerance, a hereditary defect in which transfer of ammonium ion to the urea synthesizing system is impaired, a marked elevation in plasma alanine is observed.[149] Hyperalaninemia has also been reported in a number of hyperammonemic disorders involving defects in urea cycle enzyme activity.[150–152] Alanine also functions as an intra-hepatic ammonia-binding agent when urea synthesis ceases in the anoxic liver.[153] With regard to physiologic increments in ammonia levels, the increased transfer of amino groups to pyruvate noted in exercising muscle,[12] may serve to limit the extent to which peripheral ammonia release is stimulated by muscle contraction.[121–123] These data thus indicate that the carrier role of alanine in nitrogen metabolism is of particular importance in circumstances characterized by augmented formation or inadequate disposal of ammonia.

GLUTAMINE AND GLUTAMATE METABOLISM

In the various studies cited above in which the primacy of alanine in the flux of amino acids from muscle to liver was demonstrated, the individual amino acids were measured by the column chromatographic technique.[154] With this procedure, reliable determination of plasma glutamine and glutamate is

precluded because of breakdown of glutamine on the column to glutamate and to pyrrolidine carboxylic acid.[155] Recently, Marliss et al. reported that glutamine is released from peripheral muscle and extracted by the splanchnic bed in amounts comparable to alanine.[156] While these studies reveal that glutamine is as important as alanine in nitrogen transport in resting postabsorptive man, several differences exist with regard to the relative roles of these amino acids, particularly in their function as gluconeogenic substrates.

Although net release of alanine by the gut has been demonstrated in both man[12] and experimental animals,[28] with the liver constituting the site of splanchnic uptake of alanine, a similar pattern of interorgan transfer has not been reported for glutamine. Elwyn et al. noted that glutamine is the only amino acid consistently extracted by the gastrointestinal tissues in postabsorptive dogs.[28] Furthermore, Addae and Lotspeich reported that while the gut extracted glutamine, there was a net release of this amino acid by the liver.[157] In man, recent studies have similarly shown positive arterio-portal venous differences for glutamine.[158] These data thus indicate that in contrast to alanine, the gut rather than the liver is the site of splanchnic glutamine uptake. Moreover, in diabetics in whom splanchnic uptake of alanine and other glycogenic amino acids is increased,[67] splanchnic extraction of glutamine remains unchanged from control levels.[158] Since the intestinal tract rather than the liver is the site of splanchnic glutamine disposal, and inasmuch as removal of glutamine by the splanchnic bed is not augmented in diabetes, the available data suggest that glutamine is a relatively less important endogenous gluconeogenic substrate than alanine.

Differences between alanine and glutamine with regard to nitrogen metabolism are also apparent. In contrast to alanine, during exercise an increase in arterial glutamine levels is not observed,[159] and at maximal work loads no significant output of glutamine is demonstrable across the exercising leg.[160] Furthermore, in the exercising rat a significant decrease in plasma glutamine has been reported.[130] Thus while in the resting state the contribution of glutamine to the transfer of amino groups from the periphery to the splanchnic bed is equal to that of alanine, during exercise alanine formation appears to be of greater importance.

Finally, it should be noted that Elwyn et al., studying normal dogs, recently reported that erythrocytes as well as plasma may be important in the interorgan transport of amino acids.[161] This is particularly important in the case of glutamate, whose transport by plasma and erythrocytes across the gut and liver is often in opposite directions.[161] In contrast, glycine and serine uptake by the liver in the postabsorptive state is primarily a consequence of extraction from plasma.[161] Similarly, liver uptake of alanine is due primarily to extraction from plasma rather than transfer from erythrocytes.[28]

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