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THE REGULATION OF GLUCOSE FLUXES DURING EXERCISE IN HEALTHY MALE SUBJECTS

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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Regulation of Glucose Fluxes During Exercise in Healthy Male Subjects

ABSTRACT

The present study was designed to further characterize the role of insulin and glucagon in the regulation of glucose fluxes during two hours of mild to moderate intensity exercise in postabsorptive healthy male subjects. Endogenous insulin and glucagon were suppressed by somatostatin (SRIF) infusion and the pancreatic hormones were then replaced singly or in combination to match the hormonal concentrations observed during similar exercise in control subjects. Glucose kinetics were determined using stable isotopes of glucose. The exercise protocol was sufficient to induce a 26% decrease in insulin and a 20% increase in glucagon. In all conditions, there were a 1.5- and 2-fold increase in glucose utilization and in glucose metabolic clearance rate, respectively when compared to the resting levels. Exercise alone was able to maintain the increase in glucose metabolic clearance rate despite a deficiency in insulin. Hepatic glucose production and gluconeogenesis increased to values representing 228 and 144% of the resting level, respectively. In the absence of glucagon, these increases during exercise were totally abolished. From our observations, it is concluded that during mild to moderate intensity exercise insulin was not essential for glucose uptake indicating that muscle contractions per se can stimulate glucose uptake by muscles and the increase in hepatic glucose production and gluconeogenesis are essentially dependent on the increase in glucagon. Insulin remains important, however, for maximal glucose uptake by contracting muscles and for preventing glucose overproduction and possibly hyperglycemia.

RÉSUMÉ

Utilisant une technique à doubles isotopes stables, nous avons caractérisé le rôle respectif de l'insuline et du glucagon and la régulation du métabolisme du glucose pendant un exercice de deux heures à 40% du VO_2 max chez des sujets sains. La somatostatine (SRIF) a été utilisée afin de supprimer l'insuline et le glucagon endogènes afin de les remplacer séparément (SRIF + insuline ou SRIF + glucagon) ou en combinaison (SRIF + insuline + glucagon) pour reproduire leurs concentrations observées à l'exercice chez les sujets témoins. L'insulinémie diminue à l'exercice de 26% et la glucagonémie augmente, quoique non significative, de 20% des valeurs de repos. L'exercice augmente la production hépatique de glucose seulement en présence de glucagon. La conversion de l'alanine en glucose, un indice de la gluconéogenèse augmente à l'exercice de 1.5 fois chez les témoins. Cette augmentation est abolie en l'absence du glucagon à l'exercice. Parmi toutes les conditions étudiées, en présence ou en l'absence d'insuline, l'utilisation du glucose à l'exercice augmente. En conclusion, nous suggérons que les contractions musculaires semblent être les régulateurs majeurs de l'utilisation de glucose par les muscles, et que l'augmentation de la production hépatique du glucose et de la gluconéogenèse à l'exercice prolongé d'intensité modérée sont dépendantes de l'augmentation des concentrations de glucagon. Néanmoins, une synergie semble exister entre l'insuline et les contractions musculaires pour l'expression maximale de l'utilisation du glucose.

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PREFACE

1. Statement

In accordance with the guidelines concerning thesis preparation, the candidate has taken the option of writing the experimental portions of this thesis (chapters 2 to 4, inclusive) in the form of original papers. This provision reads as follows "The candidate has the option, subject to the approval of their Department, of including as part of their thesis, copies of the text of a paper(s) submitted for publication, or the clearly-duplicated text of a published paper(s), provided that these copies are bound as an integral part of the thesis. Manuscript-style thesis must still conform to all other requirements explained in the Guidelines Concerning Thesis Preparation. Additional material (procedural and design data as well as descriptions of equipment) must be provided in sufficient detail (e.g., in appendixes) to allow clear and precise judgement to be made of the importance and originality of the research reported in the thesis. The thesis should be more that a mere collection of manuscripts published or to be published. It must include a general abstract, a full introduction and literature review, and a final overall conclusion. Connecting texts which provide logical bridges between different manuscripts are usually desirable in the interest of cohesion. Photographs or other material which do not duplicate well must be included in their original form. In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent; supervisors must attest to the accuracy of such claims at the Ph.D. Oral Defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of the different authors of co-authored papers "

2. Papers Submitted For Publication

Lavoie, C., Ducros, F., Bourque, J., Langelier, H., and Chiasson, J.-L.: Role of insulin and glucagon in the regulation of hepatic glucose production during exercise in healthy male subjects. *J. Clin. Invest.* (1993).

Lavoie, C., Ducros, F., Bourque, J., Langelier, H., and Chiasson, J.-L.: Role of insulin and glucagon in the regulation of gluconeogenesis during exercise in healthy male subjects. *J. Clin. Invest.* (1993).

Lavoie, C., Ducros, F., Bourque, J., Langelier, H., and Chiasson, J.-L.: Role of insulin in the regulation of glucose utilization during exercise in healthy male subjects. *Diabetes* (1993).

3. Role of Authors

Francine Ducros, Nurse, Research Group on Diabetes and Metabolic Regulation, was responsible for the installation of multiple intravenous cannulae and for taking the blood samples. When needed, she was the operator of the artificial pancreas (Biostator).

Josée Bourque, Research Assistant, Research Group on Diabetes and Metabolic Regulation, was responsible of the gas chromatograph mass spectrometer. She took care of the isotopic tracer samples from the evaporation step to injected them in the GS MS.

Hélène Langelier, Dietician, Research Group on Diabetes and Metabolic Regulation, was in charge of teaching the volunteers the appropriate diet, evaluating the nutritional data to calculate every subject nutritional diary.

The author, Carole Lavoie, was responsible for everything else. She had to solicit the volunteers, explain to them the study, evaluate their VO_2 max, prepare all the

equipment needed for the study (tubes, solutions, charts), neutralize and measure plasma glucose, lactate, alanine, hydroxybutyrate, glycerol and FFA. She also prepared the study scheme and every data sheet and measured all hormones: insulin, glucagon, cortisol and GH. For the isotope samples, she neutralized the samples before separating them on ion-exchange chromatography columns, lyophilized and resuspended them in methanol for Josée Bourque to inject them on the mass spectrometer. She also composed both consent forms for the VO_2 max test and the study.

4. Outline Thesis Structure

This format has allowed the inclusion, as chapters in this thesis, three original papers which will shortly be submitted for publication, one singly and two in a combined form. Chapter 1 is a general introduction, for which, the references are found at the end of the main part of the thesis. Chapters 2, 3 and 4 are the papers to be submitted. Each has its own abstract, introduction, methods and results, discussion and references, as requested by the respective journals. Chapter 5 is a general discussion of all the findings in the thesis. Finally there is an appendixes giving more details of the methodology used.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BCAA	Branched-chain amino acids
cAMP	Cyclic adenosine monophosphate
CP	Creatine phosphate
CRH	Corticotropin releasing hormone
CS	Citrate synthase
DHAP	Dihydroxyacetone phosphate
DNA	Dexoribonucleic acid
FA	Fatty acids
FFA	Free fatty acids
F-1,6-BP	Fructose-1,6-bisphosphate
F-1,6-BPase	Fructose-1,6-bisphosphatase
F-2,6-BP	Fructose-2,6-bisphosphate
F-6-P	Fructose-6-phosphate
GH	Growth hormone
GHRH	Growth hormone releasing hormone
Gi	Inhibitory guanine nucleotide regulatory protein
GLUT	Glucose transporter
GK	Glucokinase
Gp	Phospholipase guanine nucleotide regulatory protein
GPhase	Glycogen phosphorylase
Gs	Stimulatory guanine nucleotide

GTP	Guanosine triphosphate
G-1,6-BP	Glucose-1,6-bisphosphate
G-1-P	Glucose-1-phosphate
G-6-P	Glucose-6-phosphate
G-6-Pase	Glucose-6-phosphatase
G-protein	Guanine nucleotide regulatory protein
HGP	Hepatic glucose production
HK	Hexokinase
HMG-CoA reductase	3-hydroxyl-3-methylglutaryl-CoA reductase
IP_3	Inositol triphosphate
IMP	Inosine monophosphate
Km	Michaelis constant of affinity
LDH	Lactate dehydrogenase
MRC	Metabolic clearance rate
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
OAA	Oxaloacetate
PC	Pyruvate carboxylase
PDH	Pyruvate dehydrogenase
PEP	Phosphoenolpyruvate
PEPCK	Phosphoenolpyruvate carboxykinase
PFK-1	Phosphofructokinase
PFK-2/FBPase-2	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase
PK	Pyruvate kinase
Pi	Inorganic phosphate
Ra	Glucose appearance or production
Rd	Glucose disappearance or utilization

RIA	Radioimmunoassay
RNA	Ribonucleic acid
SRIF	Somatostatin
TG	Triacylglycerols
Vmax	Maximal rate
UTP	Uridine triphosphate

Please note that, throughout the thesis, VO_2 max should be read VO_2 max.

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CHAPTER 1

GENERAL INTRODUCTION

BACKGROUND

I. OVERVIEW OF INTERMEDIARY METABOLISM

Glucose enters the cell via specific transporters and is rapidly phosphorylated to G-6-P, a reaction catalyzed by HK or GK (liver and beta cell).

G-6-P can then be stored as glycogen or metabolized to pyruvate through the glycolytic pathway. The resulting pyruvate can then be converted to lactate or can be further metabolized by the citric acid cycle for aerobic ATP formation or for lipid synthesis via acetyl-CoA. On the other hand, the liver has the capability to release glucose into the circulation. This glucose can come from glycogen stores through glycogenolysis or from *de novo* glucose synthesis from lactate, amino acids and glycerol via gluconeogenesis (Fig. 1). The predominance of one of these metabolic processes will depend on several factors such as hormonal environment, cell requirement, availability of substrates, and nutritional state (Ferré and Girard, 1990).

A. Glucose Production

In the postabsorptive state, when exogenous substrates are not available, the body has to ensure an adequate fuel supply to maintain plasma glucose levels within safe limits for the central nervous system. Glucose is the only fuel for the human brain, except under starvation when ketone bodies become an alternative energy-substrate (Foster and McGarry, 1988; Stryer, 1988).

The primary role of the liver in the preservation of normoglycemia in the absence of carbohydrate intake was first recognized by Claude Bernard (1876). After an overnight fast, liver glycogen stores are the main source of glucose for the maintenance of plasma glucose concentration via glycogenolysis; but with time, glycogen stores gradually become depleted, and gluconeogenesis takes over. Thus, the liver can produce glucose

from either glycogenolysis or gluconeogenesis. The glucose released into the circulation is taken up primarily by the brain and by skeletal muscle.

Glycogenolysis is also active in skeletal muscle but lacking the hydrolytic enzyme G-6-Pase, it does not make any direct contribution to blood glucose. Skeletal muscle is strictly self-serving with regards to glycogen. Glycogen concentration is greater in the liver than in skeletal muscle, but because of the larger muscle mass, more glycogen is stored in skeletal muscle (McArdle et al., 1991).

Skeletal muscle can still contribute to blood glucose indirectly via the production of alanine and lactate. These can be recycled through the glucose-alanine and Cori cycles, respectively and provide substrates to the liver for gluconeogenesis.

a) Glycogenolysis

1º *Biochemical Pathway and Enzymes*

Glucose units in the glycogen molecule are linked by α-1,4-glycosidic bonds with branches created by α-1,6-glycosidic bonds in a ratio of 1 to 10. Glycogen is broken down by an interplay of three enzymes called glycogen phosphorylase (GPhase), transferase and debranching enzyme (α-1,6-glucosidase) which yield glycosyl residues (Geddes, 1985). GPhase catalyzes the removal of glycosyl residues as glucose-1-phosphate (G-1-P) starting from the non-reducing end of the glycogen until it reaches a terminal four residues away from a ramification. The other glycosyl residues are then transferred by transferase to the principal branch. This exposes the α-1,6-glycosidic bond to the debranching enzyme yielding one glucose molecule. G-1-P is then converted to G-6-P by phosphoglucomutase. In the liver, G-6-P can serve as a substrate for the enzyme G-6-Pase and be hydrolyzed to yield free glucose which can be released into the circulation. G-6-Pase is also present in the kidney and small intestines but is absent in the muscle as well as the brain.

2º *Regulatory Site*

GPhase is known as the rate-limiting step in glycogen degradation (Sutherland and Cori, 1951) and it is controlled by the cyclic AMP cascade (Soderling and Park, 1974; Larner, 1976). The enzyme activity is regulated jointly by humoral factors and allosteric regulators (Soderling and Park, 1974; Lowenstein and Goodman, 1978; Lowenstein, 1990; Chasiotis, 1983, 1988; van de Werve and Jeanrenaud 1987). GPhase exists in two forms: the *a* form which is phosphorylated and physiologically active, and the *b* form which is dephosphorylated and inactive (Stalmans and Gevers, 1981; Fisher, Heilmeyer and Hashke, 1971). The interconversion of GPhase *b* to *a* is induced by the phosphorylation of the enzyme by phosphorylase *b* kinase, the latter kinase being phosphorylated and activated by cAMP-dependent protein kinase. Phosphorylase *b* kinase can also be activated by calcium without phosphorylation of the enzyme. GPhase *a* can then be inactivated through dephosphorylation by protein phosphatase. Phosphatases accelerate dephosphorylation of many enzymes (Soderling and Park, 1971). Their actions are coordinated to the action of the kinases, usually these being inactivated when phosphatases are activated (Cohen, 1985a, 1985b). Phosphatases may be restricted in their actions to a single target enzyme or may act on multiple regulatory proteins. Many types of different protein phosphatases have been identified: type 1 (PP-1) and type 2 (PP-2A, PP-2B, and PP-2C) (Ingerbritsen, Foulkes and Cohen, 1983). Among them, a different sensitivity to phosphatase inhibitor protein have been found.

The allosteric regulators involved in the control of GPhase are Ca^{2+}, AMP, IMP, G-6-P and Pi. The *a* or active phosphorylated form of GPhase is not influenced by either the absence of AMP or of ATP and G-6-P. The *b* or inactive dephosphorylated form, on the other hand, can be activated by AMP, IMP and Pi without

phosphorylation. However, these allosteric activations of the enzyme are easily inhibited by ATP and G-6-P. Glucose is also known to directly inhibit GPhase *a*.

During exercise, Pi derived from creatinine phosphate (CP) may be closely linked to muscular glycogenolysis (Chasiotis, 1988; Chasiotis, 1983). With prolonged exercise, the augmentation of the intracellular concentration of Pi, IMP and AMP could activate GPhase *b*. Recently, we showed that muscle glycogen breakdown could be maintained during muscle contractions despite an increased interconversion of GPhase *a* to *b*, suggesting that GPhase *b* was activated through allosteric activators without phosphorylation of the enzyme (Lavoie, Péronnet and Chiasson, 1992).

Some years ago, it was proposed by Lowenstein (1972, 1990) that the exercise-induced increase in muscle IMP could serve to maintain glycogenolysis by activating GPhase *b* (Aragon, Tornheim and Lowenstein, 1980). The role of IMP, however, still remains unclear since glycogenolysis wanes even at high IMP concentrations when fatigue develops (Tullson and Terjung, 1991). On the other hand, the elevated Pi levels observed during exercise seem to play a primary role in the maintenance of glycogen breakdown during muscle contractions (Hultman and Spriet, 1986). The rise in AMP during exercise is controversial and it is very unlikely that it plays an important role in glycogen breakdown in contracting muscles. Hormonal regulation of glycogenolysis is mediated through phosphorylase kinase by cAMP-dependent protein kinase and/or calcium. Therefore, glucagon and catecholamines, can act through β-adrenergic by increasing cAMP and by activating cAMP-dependent protein kinase whereas stimulation from vasopressin and angiotensin II, can act through α_1-adrenergic stimulation by mobilizing calcium and by activating phosphorylase kinase agonist which in turn phosphorylate and activate GPhase and stimulate glycogenolysis (Jeanrenaud, 1987).

b) Gluconeogenesis

Gluconeogenesis is an energy-demanding process (Pilkis et al., 1988) that results in the formation of glucose or glycogen from lactate, pyruvate, glycerol and glucogenic amino acids (Exton, 1972; Cahill, 1986). The liver and kidney are the only organs with the capability to synthesize glucose from non-carbohydrate precursors. The liver is the major site of gluconeogenesis with the kidney becoming important only during starvation and acidosis (Exton, 1972). Gluconeogenesis plays a crucial role in energy metabolism. First, it provides glucose for the body when carbohydrate intake is limited and body glycogen stores are depleted. Second, it also masters the recycling of lactate and glycerol produced under basal conditions as well as during exercise and in response to sympathetic activity (Exton, 1972). Third, it serves in the metabolism of amino acids coming from the alimentary tract or released from muscle and other tissues during protein breakdown (Exton, 1972; Ruderman, 1975). And finally, gluconeogenesis provides ammonium (NH_3) to the kidneys to counteract acidosis particularly during prolonged starvation.

1° *Biochemical Pathway and Enzymes*

Gluconeogenesis is not a simple reversal of glycolysis. In fact, three glycolytic enzymes have to be bypassed because they catalyze irreversible steps: these are HK, PFK-1 and PK. They are bypassed respectively by G-6-Pase, F-1,6-BPase, and PC plus PEPCK which catalyze the flow of metabolites in the direction of gluconeogenesis (Cahill, 1986; Stryer, 1988; Zubay, 1988).

The newly formed glucose resulting from those metabollic steps can either be stored as glycogen or released into the circulation are as follows. Lactate, alanine, serine and glycine are first converted to pyruvate in the cytosol. Pyruvate gains entry into the mitochondrion in exchange for OH^- via a pyruvate carrier mechanism located in the inner membrane of the mitochondrion (Stryer, 1988; Zubay, 1988). Pyruvate is converted

to either OAA by PC (representing the major route) or to acetyl-CoA by PDH (Exton, 1972). The PDH step represents a point of no return for glucose carbon in carbohydrate metabolism (Ferré and Girard, 1990). OAA, however, can serve as an intermediate in the citric acid cycle or as an intermediate in gluconeogenesis (Stryer, 1988). A high concentration of acetyl-CoA indicates that there is a need for more OAA. So, if there is a surplus of ATP, OAA will be diverted into the gluconeogenic pathway. If there is a need for ATP, however, OAA will enter the citric acid cycle to complete oxidation (Stryer, 1988). All the enzymes involved in gluconeogenesis are cytoplasmic except for PC which is a mitochondrial enzyme. OAA is reduced to malate for its transport by a carrier in exchange for Pi into the cytosol, where it is reoxidized to OAA by a NAD^+ - linked malate dehydrogenase (Stryer, 1988). PEPCK then converts cytoplasmic OAA to PEP which in turn is converted to F-1,6-BP by the reversible glycolytic enzymes. The next step, the interconversion of F-1,6-BP to F-6-P by F-1,6-BPase is a key point in the regulation of glycolysis and gluconeogenesis. More details of this crucial regulatory step will be discussed in the next section. Finally, G-6-P is converted to glucose by G-6-Pase. As a gluconeogenic precursor, glycerol enters at the level of the triose phosphate: DHAP after reacting with glycerokinase to form glycerol-1-phosphate and being oxidized by α-glycerophosphate dehydrogenase (Stryer, 1988).

2º *Regulation Sites and Allosteric Regulators*

Several sites for gluconeogenic regulation have been described in animals (Cherrington and Vranic, 1986). Control can be exerted in the periphery from where the substrates are supplied, at the liver where substrates have to be extracted or within the hepatocyte where the substrates are converted to new glucose (Cherrington and Vranic, 1986). Only the last control site within the hepatocyte will be discussed. Recently, it has been shown by Jahour and colleagues (1990) that a rise in gluconeogenic precursor supply alone will not induce glucose production through gluconeogenesis, indicating that

factors which regulate directly the rate-limiting enzyme activities of glucose production are the major determinants of the rate of new glucose formation. Thus, an increased supply in gluconeogenic precursors will only result in an increase in glucose production if there has been a prior stimulation of the enzymatic system (Jahour et al., 1990).

The first regulatory site in the gluconeogenic pathway is at the pyruvate-PEP cycle. PK has to be inhibited and PC as well as PEPCK have to be activated to allow for gluconeogenesis. PK is stimulated by F-1,6-BP and inhibited by ATP, alanine, FFA and acetyl-CoA while PC is inhibited by ADP and stimulated by acetyl-CoA. PEPCK is also inhibited by ADP. In brief, the flow of pyruvate to PEP and gluconeogenesis is hastened when the cell is rich in fuel molecules and ATP. It is important to point out, that the step from pyruvate to acetyl-CoA is most critical in controlling the balance between carbohydrates and lipids, because once pyruvate is oxidized to acetyl-CoA, it cannot be retrieved for glucose formation (Cahill, 1986) (Fig. 2).

Glucagon and β-adrenergic agonists phosphorylate PK resulting in decreased enzyme activity and favoring gluconeogenesis (Claus et al. 1983; Pilkis et al. 1985, 1988, 1990; Pilkis and Claus, 1991).

The synthesis of PEPCK will be increased *in vivo* by glucagon, glucocorticoids, and epinephrine and decreased by insulin and glucose (O'Brien and Granner, 1990). Therefore, glucagon, glucocorticoids and catecholamines will stimulate gluconeogenesis while insulin and glucose will inhibit the metabolic process at the pyruvate-PEP step.

One of the major regulatory sites of gluconeogenesis is at the level of F-1,6-BP and F-6-P (Fig. 3). Gluconeogenesis and glycolysis are coordinated so that one

pathway is favor over the other (Hue and Bartrons, 1984; Pilkis and El-Maghrabi, 1986; Hue and Rider, 1987; Pilkis et al., 1990; Pilkis and Claus, 1991).

F-2,6-BP is the master switch regulating the flow between gluconeogenesis and glycolysis in the liver (Fig. 3). This metabolite is derived from F-6-P and is the most important allosteric regulator that stimulates PFK-1 and inhibits F-1,6-BPase thus favoring glycolysis while inhibiting gluconeogenesis. By decreasing the level of this potent regulator of PFK-1, gluconeogenesis is automatically activated while glycolysis is inhibited. The enzyme PFK-2/FBPase-2 is a unique bifunctional enzyme which regulates both the synthesis and degradation of F-2,6-BP. The activity of the enzyme is under the control of a cAMP-dependent protein kinase which catalyzes phosphorylation of the bifunctional enzyme resulting in stimulation of the phosphatase activity. The phosphorylated form is active as a phosphatase while the dephosphorylated form is active as a kinase (Pilkis et al., 1990). The phosphorylated form of the enzyme will convert F-2,6-BP to F-6-P decreasing the level of F-2,6-BP while the dephosphorylated form will convert F-6-P to F-2,6-BP thus increasing the levels of this metabolite. Since F-2,6-BP stimulates PFK-1 and inhibits F-1,6-BPase, increased levels of this metabolites will stimulate glycolysis and decreased levels will favor gluconeogenesis (Pilkis et al., 1990). F-2,6-BP is a potent allosteric activator of PFK-1 with a Ka of 0.05 μM and is almost a hundred times more potent than F-1,6-BP in activating PFK-1. ATP as well as citrate will tend to inhibit PFK-1. F-2,6-BP, however, can overcome ATP inhibition of the enzyme and work synergistically with AMP to activate it. The metabolite is also a powerful competitive inhibitor of F-1,6-BP with a Ki around 0.5 μM. F-2,6-BP also enhances the inhibition of the PFK-1 enzyme by AMP (Pilkis and El-Maghrabi, 1986).

In the liver, glucagon activates cAMP-dependent protein kinase which phosphorylate the bifunctional enzyme resulting in increased phosphatase activity and

consequently lead to a decreased in the levels of F-2,6-BP thus favoring gluconeogenesis. Insulin has the opposite effect resulting primarily from a decrease in cAMP concentration and finally stimulation of glycolysis (Table I). But the exact mechanisms of insulin implicated at the level of the bifunctional enzyme are still unclear (Unger and Foster, 1992).

Hue et Rider (1987) have suggested a relationship between hepatic F-2,6-BP concentration and several physiological conditions. In a gluconeogenic state such as fasting, low F-2,6-BP are associated with increased levels of glucagon, β-adrenergic agonists, FFA and cAMP. Moreover, muscular exercise through an increase levels in cAMP seems to be involved in the decrease of hepatic F-2,6-BP. On the other hand, high F-2,6-BP (5 and 20 nmol/g) are associated with a glycolytic state and are the result of a glucose load, insulin administration or refeeding (Hue and Rider, 1987). In skeletal muscle and heart, F-2,6-BP is also involved in the control of glycolysis under the effect of epinephrine and insulin. These are believed to be the major control elements for switching from glycolysis to gluconeogenesis (Hue and Rider, 1987). On the other hand, it has been shown in the muscle that epinephrine stimulate glycolysis, this resulting from a cAMP-induced formation of F-2,6-BP. However, the exact proteins involved in this process are still to be characterized (Hue and Rider, 1987b).

Lastly, the glucose/G-6-P cycle is another point of regulation. HK is allosterically inhibited by G-6-P (Stryer, 1988). G-6-Pase regulation depends mainly on activation by glucagon, glucocorticoids and catecholamines and inactivation by insulin (Harper, Rodwell and Mayes, 1982; van de Werve and Jeanrenaud, 1987).

Blood glucose concentration will also determine the balance between hepatic glucose production and peripheral utilization. Because glucose enters hepatocytes by passive diffusion through GLUT2 transporter molecules down its concentration

gradient, the increase in blood glucose concentration will promote glycolysis while low blood glucose concentration will enhance gluconeogenesis. GK with a higher Km for glucose when compared to HK will further facilitate the diffusion of free glucose through the cell membrane and make the liver highly receptive to an increased blood glucose concentration.

3º *Gluconeogenic Precursors*

The principal substrates for liver gluconeogenesis are lactate, glycerol and the glucogenic amino acids (Brosnan, 1982). *In vivo*, alanine and glutamine, are the two most important glucogenic amino acids (Ruderman and Berger, 1974; Ruderman, 1975). Glutamine, however, is preferentially taken up by the gut instead of the liver, resulting in a lower availability for gluconeogenesis. Therefore, it is not as important as alanine (Felig and Wahren, 1974). Felig and Wahren (1971) showed that alanine synthesized from the transamination of pyruvate in skeletal muscle was increased during exercise because of the increased availability of pyruvate.

Looking at all the potential glucogenic precursors, Aikawa and colleagues (1972) compared various precursors of hepatic gluconeogenesis *in vivo* and found that net production of glucose rises concomitantly with an increase in the plasma concentration of alanine, serine or lactate and that glycerol, aspartate and glutamate are less effective at high plasma concentrations than at physiological plasma concentrations.

It has, recently, been shown in healthy postabsorptive male subjects that muscle was the major source of plasma alanine (~70%) and lactate (~40%) and that yet unknown factors responsible for their release from muscle could play an important role in the regulation of hepatic gluconeogenesis (Consoli et al., 1990).

All gluconeogenic precursors reach the liver in subsaturating concentrations. It was therefore suggested that any change in their concentrations could affect the rate of gluconeogenesis; an increase in gluconeogenic precursors would increase gluconeogenic rate and a decrease in the arrival of precursors would slow down gluconeogenesis (Pilkis et al., 1990). However, as mentioned previously, Jahour et al. (1990) have shown that this was not the case and that a rise in gluconeogenic precursors was not enough to elicit an increase in the rate of gluconeogenesis. For this to occur, prior enzymatic stimulation is required.

Lactate. Some decades ago, Cori proposed a cycle for glucose recycling consisting of liver glycogen, blood glucose, muscle glycogen and blood lactate (Cori, 1931, 1981). He pointed out that some of the glucose and glycogen utilized by peripheral tissues was not oxidized, but was released as lactate and pyruvate which were reconverted to glucose in the liver and then cycled back to the periphery (Cori, 1931). The tissues contributing to the Cori cycle are red cells, skin, renal medulla (which derive energy principally from anaerobic glycolysis), skeletal muscle and brain where fasting results in an increased formation of lactate and pyruvate due to the inhibition of pyruvate oxidation (Reichard et al., 1963). During exercise, lactate is formed in excess, passes into the circulation and becomes available to the liver for conversion to glucose (Cori, 1981). The newly formed glucose can be released into the circulation or stored as glycogen in the liver. The latter is referred to as the indirect pathway of glycogen synthesis (Radziuk, 1989ab; Pilkis et al., 1985). Thus, the Cori cycle is a means whereby the lactate produced in erythrocytes and exercising muscles can be recycled into glucose (Pilkis et al., 1985, 1990). This metabolic process first described by Cori (1931) is of particular importance in maintaining glucose homeostasis under various situations such as starvation and/or exercise.

Amino acids. Of all the glucogenic amino acids, alanine is quantitatively the most important (Felig et al., 1969, 1970ab; Felig and Wahren, 1971). Splanchnic extraction of alanine can reach approximately 41% in the postabsorptive state as measured by the arteriovenous difference technique (Felig et al., 1969). A substantial amount of this alanine is formed in skeletal muscle by the transamination of pyruvate. As for lactate, alanine can be converted back to glucose in the liver by way of gluconeogenesis (Felig et al., 1969, 1970ab; Felig and Wahren, 1971, 1974; Felig, 1973).

The glucose-alanine cycle first described by Felig (1970b, 1973) can be viewed as a way for the glucose-derived pyruvate to be transaminated in muscle to form alanine which is taken up by the liver where its carbon skeleton can be recycled into glucose. This recycling is also important in respect to nitrogen metabolism. Alanine is a safe non-toxic way to transfer amino groups derived from amino acid metabolism in muscle to the liver for disposal (Felig, 1973; Felig and Wahren, 1974; Pilkis et al., 1990).

Gluconeogenesis also contributes significantly to the use of amino acids, whether they are absorbed from the alimentary tract or released during protein breakdown (Pilkis et al., 1990). Oxidation of BCAA such as leucine, isoleucine and valine can provide amino group for *the novo* formation of alanine and glutamine (Elperin and Rolleston, 1990; Goldberg and Chang, 1978). Transamination of the BCAA passes through an α-ketoglutarate to form glutamate, which may either supply its amino group to pyruvate to form alanine or be converted to glutamine by the addition of ammonia (Goldberg and Chang, 1978; Kettelhut et al., 1988). Exercise in general leads to an acceleration of the breakdown of the BCAA (Wagenmakers, 1989). The intestinal tract can also extract glutamine, glutamate and aspartate for the production of alanine through transamination. Alanine from both skeletal muscle and intestines is channeled to the liver to generate glucose via gluconeogenesis (Goldberg and Chang, 1978; Kettlehut et al., 1988). Alanine and glutamine are formed *de novo* in great quantity in skeletal muscle

(Ruderman, 1975). Their release is a means to get ride of toxic NH_3 from amino groups: alanine carbon skeleton as mentioned earlier is the preferential substrate for hepatic gluconeogenesis while the skeleton of glutamine will participate preferentially in renal gluconeogenesis or serve as energy-substrate for various cells (gastrointestinal epithelia, fibroblasts and reticulocytes) (Kettlehut et al., 1988).

In summary, both cycles (Cori and alanine-glucose cycle) play an important role in energy-substrate conservation by recycling carbons from glycogen within the exercising muscle to glucose in the liver and the glucose thus formed can be reutilized by working muscle when muscle glycogen stores decrease (Péronnet et al., 1983).

Glycerol. Glycerol is released together with three FFA when TG are hydrolyzed by lipases. In the liver, glycerol formed by lipolysis is phosphorylated, oxidized and then isomerized to glyceraldehyde-3-phosphate. This intermediate can take either the glycolytic or the gluconeogenic pathway. As glycerol enters the gluconeogenic pathway at the triose phosphate level, it is not exposed to the rate-limiting step between pyruvate and phophoenoylpyruvate (Exton, 1972). As such, it should be theoretically the favorite substrate for gluconeogenesis. In the postabsorptive state approximately 40% of glycerol and alanine are converted to glucose (Winkler et al., 1970; Chiasson et al., 1977; Jahour et al., 1990). Using tracer methods in postabsorptive dog, Gauthier et al. (1983) found that only 28% of the carbon atoms from glycerol were converted to glucose through gluconeogenesis.

Mobilization of fat stores from adipocytes will act as an energy source for gluconeogenesis and as a modulator of major enzymes: FA will enhance F-1,6-BPase and will reduce both PFK-1 and PK activities through citrate accumulation (Taylor and Halperin, 1973). Increased FFA levels have also been shown to decrease liver

glycogenolysis. As such, lipolysis can play an important role in regulating hepatic glucose production as reported by Clore et al. (1991ab). The relative participation of gluconeogenesis and glycogenolysis to hepatic glucose metabolism may be modulated by the levels of gluconeogenic precursors and substrates such as FFA without affecting the total amount of glucose produced by the liver.

B. Glucose Utilization

a) Glucose Transport

1° *Description*

Before glucose can be channeled into any metabolic pathway, it has to be taken up by the cell. Plasma membrane being impermeable to polar molecules such as glucose, the transport of this mandatory nutrient is done by an integral plasma membrane protein that binds glucose and transfers it across the lipid barrier; these are called glucose transporters (Simpson and Cushman, 1986; Kahn, 1992) (Fig. 4). This process differs in different tissues, both with respect to the type of transporters and to energy dependence (Wallberg-Henriksson 1987). So far, eight different kinds of glucose transporters have been identified (Bell et al., 1990; Kahn, 1992). Generally, they fall into one of two types of transporters: Na^+-glucose co-transporter or facilitative glucose transporter. In the former, glucose is transported against its concentration gradient by coupling its uptake with the uptake of Na^+ that is being transported down its concentration gradient. This type of glucose transporter is found in specialized epithelial (brush border) cells of the small intestines and proximal tubules of the kidneys (SGLT 1) and is an energy dependent process (Bell et al., 1990). In the latter, the transport of glucose is accomplished by integral membrane proteins present on all cell surfaces which transport glucose down its concentration gradient by passive transport. Facilitative glucose transporters are expressed by most if not all cells: erythrocytes (GLUT1), brain cells (GLUT3),

hepatocytes and β cells (GLUT2), muscle cells and adipocytes (GLUT4) and cells of the small intestines (GLUT5) (Bell et al., 1990). Table II describes the various glucose transporters identified in man (Bell et al., 1990). Among all of the glucose transporters, only GLUT4 seems to be hormonally sensitive (Joost and Weber, 1989). GLUT4 is insulin-sensitive and is found in skeletal and cardiac muscles as well as in adipose tissue (Simpson and Cushman, 1986; James et al., 1988).

For the purpose of the present work, we will limit our discussion to GLUT2, GLUT1 and GLUT4. Excellent reviews on the subject have been published (Thorens et al., 1990; Mueckler, 1990; Kahn, 1992; Kahn and Flier, 1990; Pilch, 1990). Hepatocytes and pancreatic β-cells express the GLUT2 facilitative transporter isoform. GLUT2 has a high Km for glucose (~20-60 mM); this is the concentration of glucose at which the uptake velocity of glucose is at half the Vmax. GLUT2 also has a high Vmax which is the maximum velocity observed for a fixed amount of carriers (Kasanicki and Pilch, 1990; Thorens et al., 1990). This explains why glucose uptake by the cells expressing the GLUT2 isoform augments proportionally to the increase in blood glucose levels (Bell et al., 1990). Therefore, in tissues expressing GLUT2, glucose transport does not become rate-limiting (Kahn, 1992). This isoform also allows β-cell to react to large increases in blood glucose levels by releasing insulin so that the glucose can be taken up preferentially by peripheral tissues (muscle and adipose tissue) rather than the liver (Bell et al., 1990). Moreover, because of its high Km (low affinity for glucose), the GLUT2 isoform in the liver allows both glucose influx and efflux corresponding to the dual role of the liver in glucose production and glucose utilization (Kahn, 1992).

GLUT1 and GLUT4 have been identified in adipocytes and skeletal muscles the two main insulin-responsive tissues (Klip and Pâquet, 1990). GLUT1 is mainly located in the plasma membrane and is believed to be responsible for basal glucose transport in the resting unstimulated state. On the other hand, GLUT4 is

predominantly located in the cytoplasm and is responsible for the increase in glucose uptake after insulin stimulation (Klip and Pâquet, 1990). Both GLUT1 and GLUT4 can be translocated to the plasma membrane by insulin stimulation but in different proportions (Kahn, 1992; Joost and Weber, 1989). Insulin can activate both the translocated glucose transporters and the transporters residing in the plasma membrane (Joost and Weber, 1989). These glucose transporters migrate from an intracellular pool to the plasma membrane in response to insulin (Klip et al., 1986; Young et al., 1986; Wallberg-Henriksson, 1987; Constable et al., 1988; Wallberg-Henriksson et al., 1988; Fushiki et al., 1989; Guerre-Millo et al., 1989; Klip et al., 1989; Bonen et al., 1990; Bourey et al., 1990; Gulve et al., 1990; Kern et al., 1990), exercise (Holloszy et al., 1986; Wallberg-Henriksson, 1987; Bonen et al., 1989; Douen et al., 1989; King et al., 1989; Sternlicht et al., 1989; Cartee et al., 1989, 1990; Goodyear et al., 1990; Young et al., 1991) and hypoxia (Cartee, 1991).

2° *Regulatory Sites*

Various effectors have been found to influence glucose transport in skeletal muscle (Wallberg-Henriksson, 1987). Most of the studies on the regulation of glucose transporters were done in adipocytes. More recently, however, more work has been done investigating glucose transporters directly involved in skeletal muscle (Wallberg-Henriksson, 1987; Goodyear et al., 1990; King et al., 1989; Klip and Pâquet, 1990; Ploug et al., 1992). Insulin brings about the insertion of previous intracellularly stored glucose transporters into the plasma membranes concomitantly with some qualitative alterations of the glucose transporter. Insulin increases the translocation of the pool of transporters associated with microsomal membranes and also activates them, so that each transporter can take more glucose (Joost, Weber and Cushman, 1988; Joost and Weber, 1989). The maximal effects of muscle contractions and insulin on glucose transport are additive in mammalian skeletal muscle, providing evidence that exercise and

insulin stimulate glucose transport by separate pathways (Zorzano et al., 1986; Constable et al., 1988; Wallberg-Henriksson et al., 1988; Sternlicht et al., 1989; Young et al., 1991). In contrast to the effect of insulin and other hormones on amino acid transport, the effects of insulin on glucose transport seem to be independent of the changes in cAMP levels and happen very rapidly (Kahn, Smith and Chin, 1992). On the other hand, it has been shown that epinephrine or β-agonists inhibit insulin stimulated glucose transport in adipose tissue and skeletal muscle (Joost and Weber, 1989; Kasanicki and Pilch, 1990; Megeney et al., 1991). It has been postulated that catecholamines could weaken the activity of the insulin receptor kinase because of a reduction in the sensitivity of adipocytes to insulin and could decrease the activity of the glucose transporter *per se* (Joost et al., 1986).

Hypoxia is also a potent stimulator of glucose entry in muscle fibers (Wallberg-Henriksson, 1987; Katz and Sahlin, 1989; Sahlin, 1990; Cartee et al., 1990, 1991).

It is not known yet if epinephrine inhibition of glucose uptake is done directly at the level of glucose transport or through an increase of glucose intermediates causing an inhibition of HK. It has been shown recently by Raz et al. (1991), that epinephrine inhibits insulin-mediated glucose utilization, at least partly, from an increase in G-6-P (which inhibits HK) with a concomitant stimulation of glycolysis from G-1,6-BP; F-6-P; and F-1,6-BP mediated activation of PFK-1.

Recently, it has been proposed by Youn et al. (1991) that an increase in cytosolic Ca^{2+} concentration too small to initiate muscle contraction could induce an increase in glucose transport activity in skeletal muscle.

b) Glycogen Storage

After entry into the muscle cell, glucose can be metabolized via non-oxidative or oxidative pathways. Non-oxidative glucose disposal takes place through the storage of glucose as glycogen and through non-oxidative glycolysis leading to the formation of lactate. The oxidative pathway refers to the channeling of pyruvate through the citric acid cycle (Koivisto and Yki-Järvinen, 1990). Glycogen formation is the principal non-oxidative pathway for glucose metabolism, as suggested by a good correlation between the rate of non-oxidative glucose disposal and muscle glycogen synthesis (Koivisto and Yki-Järvinen, 1990).

1° *Biochemical Pathway and Enzymes*

Once inside the cell glucose is phosphorylated into G-6-P by HK and GK in the liver. From that crossroad, it can be diverted into glycogen for storage. The rate-limiting step is catalyzed by GSase. This enzyme exists under two interchangeable forms via phosphorylation (inactive D form) and dephosphorylation (active I form). GSase is phosphorylated by a number of various kinases including phosphorylase kinase, cAMP-dependent protein kinase, calmodulin-dependent kinase, GSase kinase 3, 4, and 5, and casein kinase-1 (Embi et al., 1981; Cohen et al., 1982; Picton et al., 1982; Sheorain et al., 1984, 1985a, 1985b; Kuret et al., 1985). GSase is phosphorylated on different sites now well identified and this is done by specific kinases. The cAMP-dependent protein kinase is responsible for the phosphorylation of sites 1a, 1b and 2; GSase kinase 3 for sites 3a,3b, and 3c; GSase kinase 4 and 5 for sites 5 and 2 respectively; phosphorylase kinase for site 2, calmodulin-dependent protein kinase for sites 1b and 2, and finally casein kinase-1 probably for site 6 (Sheorain et al., 1984). These phosphorylated sites have been shown to reduce the enzymatic activity of the enzyme (Parker et al., 1982; Sheorain et al., 1982a, 1982b; Parker et al., 1983; Cohen, 1985). G-6-P is isomerized to G-1-P to react with UTP to form uridine diphosphate-glucose (UDP-glucose), that will serve as the

glucose donor in the biosynthesis of glycogen. This reaction is catalyzed by UDP-glucose pyrophosphorylase. Glucose from UDP-glucose is transferred to the hydroxyl group at a C-4 terminus of glycogen to form an α-1,4-glycosidic linkage through the action of GSase. Recently, a new primer termed glycogenin has been discovered by Cohen et al. (1991). Glycogenin forms a complex with GSase so that its elongation can be catalyzed by the enzyme.

2° ***Regulatory Sites***

GSase activity is regulated by covalent modification of the enzyme through phosphorylation/dephosphorylation which transforms the active dephosphorylated GSase I into an inactive phosphorylated GSase D (Soderling and Park, 1974). The latter depends on a higher level of G-6-P for its activity whereas the former is active in the presence or absence of G-6-P. From the different kinases enumerated previously, catecholamines and glucagon, insulin and calcium-dependent hormones can regulate GSase activity by controlling its degree of phosphorylation (Chiasson et al., 1980, 1981; Uhing et al., 1981; Larner et al., 1982; Parker et al., 1982, 1983; Kahn, 1985; van de Werve and Jeanrenaud, 1987). GSase activation through its dephosphorylation is catalyzed by several cytosolic protein phosphatases (Khatra et al., 1980; Soderling and Park, 1974; Ingerbritsen and Cohen, 1983ab; Cohen, 1985). Recently, Raz et al. (1991) reported using muscle biopsies from human quadriceps femoris before and after euglycemic hyperinsulinemic clamp that epinephrine inhibits insulin-mediated glycogenesis through inactivation of GSase and activation of glycogenolysis through stimulation of GPhase.

c) Glycolysis

Glycolysis is a mean whereby, every cell in the body can derive energy rapidly in the presence or absence of oxygen. Muscle and hepatic glycolysis are considered here.

1° Biochemical Pathway and Enzymes

Glycolysis converts glucose to pyruvate with the concomitant production of ATP. In the presence of oxygen, pyruvate enters the mitochondrion, where it is completely oxidized to CO_2 and H_2O in the citric acid cycle. Under anaerobic conditions, pyruvate is converted to lactate (Stryer, 1988). Glycolysis is a cytosolic pathway that can be controlled at four different steps: glucose transport and the steps catalyzed by HK, PFK-1 and PK. The rate-limiting step for glycolysis is at the level of PFK-1. Substrates for glycolysis are derived either from circulating blood glucose or from previously stored glycogen; thus glycolysis is an absolute carbohydrate pathway. Glucose is phosphorylated by HK or GK (in the liver and in the pancreatic β cells) to G-6-P (Ferré and Girard, 1990). G-6-P is isomerized to F-6-P by phosphoglucose isomerase before it is phosphorylated by PFK-1 to F-1,6-BP, the first committed step of glycolysis. F-1,6-BP is then cleaved by aldolase to 3-carbon compounds: DHAP and glyceraldehyde 3-phosphate. Glyceraldehyde 3-phosphate dehydrogenase catalyzes the conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. The next step is the first ATP-generating step in glycolysis where 1,3-bisphosphoglycerate reacts with phosphoglycerate kinase to form 3-phosphoglycerate. This metabolite is then rearranged to 2-phosphoglycerate by phosphoglyceromutase and that compound is further converted to PEP by enolase. Finally, PEP is converted to pyruvate by PK with the generation of another ATP molecule. The net amount of ATP formed from one glucose molecule in the glycolytic pathway is two ATPs, because two of the four ATPs are lost in the initial phosphorylation of glucose (McArdle et al., 1991). When mitochondrial activity is insufficient, pyruvate is reduced by LDH to lactate in order to oxidize NADH (Brooks and Fahey, 1985). LDH is in constant competition with mitochondria for pyruvate. Two types of LDH exist, muscle (M) and heart (H) which share some similarities. The M-

LDH possesses a high affinity for pyruvate while the H-LDH exhibits a lower affinity (Brooks, 1985; Stryer, 1988).

2° Regulatory Sites

PFK-1 is the key enzyme in the regulation of glycolysis while HK and PK participate only to set the pace of glycolysis (Stryer, 1988). The control is exerted at the level of the irreversible reactions thus at the level of HK, PFK-1 and PK (Hue and Rider, 1987).

Most of the allosteric regulators are the same as those involved with gluconeogenesis, but they favor opposite reactions. These have already been discussed in the section on the regulation of gluconeogenesis (see section A. B) 2°)).

The hepatic PFK-1 is inhibited by large levels of ATP, which decrease its affinity for F-6-P (Fig. 5). This inhibition, however, is reversed by AMP. Thus a decrease in the ratio of ATP/AMP activates the enzyme. Citrate and H^+ also inhibit the enzyme. F-2,6-BP is the most powerful activator of PFK-1. F-2,6-BP is (Fig. 3) formed from F-6-P by a bifunctional enzyme as previously discussed. Glucagon, β-adrenergic agonists and cyclic AMP will decrease F-2,6-BP concentration through a cyclic AMP-dependent inactivation of PFK-1-2 kinase part of the bifunctional enzyme (phosphatase counterpart of the bifunctional enzyme). On the other hand, insulin, a glucose load and α-adrenergic agonists will increase liver F-2,6-BP concentration, thus favoring glycolysis (Hue and Rider, 1987; Raz et al., 1991).

Muscle PFK-1-2/FBPase-2 is more a phosphatase than a kinase, acting like the phosphorylated form of the bifunctional liver enzyme (Hue and Rider, 1987). Epinephrine and insulin stimulate glycolysis and an increase in F-2,6-BP both in heart and skeletal muscle (Hue et al., 1982). The skeletal muscle enzyme activity does not

seem to be influenced by phosphorylation from cAMP-dependent protein kinase (Hue and Rider, 1987). Thus, the epinephrine-induced increase in F-2,6-BP in muscle could result from allosteric modification of hexose monophosphates through cAMP-dependent stimulation of muscle glycogenolysis (Winder et al., 1991). Insulin, on the other hand, increases F-6-P concentration through an activation of glucose transport (Hue et al., 1982). It is not known yet, if insulin exerts any effect on the activity of skeletal muscle PFK-2/FBPase-2.

Citrate will also act as an allosteric regulator by potentiating the inhibition of ATP on PFK-1 activity signaling that the need for building blocks are filled. Citrate is a potent inhibitor of PDH and PFK-1 (Taylor and Halperin, 1973). Two stimulatory actions of citrate are known: the first one is on the key enzyme of gluconeogenesis F-1,6-BPase and the other one is on FA synthesis by enhancing acetyl-CoA carboxylase activity. Acetyl-CoA inhibits thiolase and stimulates PC activities.

Citrate inhibition of PFK-1 will lead to an accumulation of F-6-P and G-6-P, resulting in the inhibition of HK. This inhibition of HK was thought to be a mean to spare glucose uptake but evidence for that mechanism is still far from resolved (Ferré and Girard, 1990). This FFA-glucose interaction with regards to glucose utilization is known as the Randle cycle (Randle et al., 1963, Randle, 1964ab).

PFK-1 regulation under *in vivo* conditions is still poorly characterized. *In vitro*, however, PFK-1 is subject to multiple regulation; being activated by AMP, ADP, P_i, F-6-P, F-1,6-BP, F-2,6-BP, G-1,6-BP and cAMP and inactivated by H^+, ATP and citrate (Katz and Sahlin, 1989). More regulators emerge every day but their implications in mammalian skeletal muscle remain unclear.

The second enzyme to be regulated is muscle HK and it is inhibited by its product G-6-P so an inhibition of PFK-1 will result in an accumulation of F-6-P and G-6-

P with inhibition of HK (Katz et al., 1991a). However, GK in the liver is not inhibited by G-6-P, because of its high Km for glucose, and therefore, is operative only when glucose is abundant such as in the postprandial period, when the liver takes up glucose (Stryer, 1988). GK is there mainly to provide G-6-P for the synthesis of glycogen. The elevated Km of GK in the liver gives the brain and muscle priority on glucose when its supply is scarce (Stryer, 1988).

The phosphorylated form of glucose, G-6-P, is the stronger inhibitor of HK. It has been shown in healthy male subjects, that hyperglycemia resulted in a marked accumulation of free glucose inside the muscle (Katz et al., 1991b), suggesting that HK activity catalyzing the phosphorylation of glucose, was not able to follow the rate of glucose uptake. Katz and colleagues (1991b) suggested then, that glucose transport could have exceeded HK capacity to phosphorylate glucose in healthy subjects during hyperglycemia.

Lastly, PK which catalyzes the last irreversible step in glycolysis directs the outflow from this pathway. Three forms of isozymes exist in mammals: the L-PK dominates in liver, the M-type in brain and muscle and the A type for all other tissues. The only difference between the isozymes is in their regulation. The L-type isozyme is allosterically inhibited by ATP and alanine while it is activated by F-1,6-BP. The L-isozyme, but not the M-type, is also controlled by phosphorylation/dephosphorylation (Blair, 1986).

The A-type is in between the M- and L-type in its sensitivity to be controlled by reversible phosphorylation (Blair, 1986). Glucagon secretion in response to low blood glucose will increase the proportion of phosphorylated PK in the less active form. It has been shown that a variety of hormones will inhibit hepatic PK through either an increase in cAMP or an increase in Ca^{++} resulting in an activation of Ca^{++}/cAMP-

protein kinase (Pilkis et al., 1990). Phosphorylation of L-PK by cAMP-dependent protein kinase will result in its inhibition (Blair, 1986; Evans et al., 1986). Allosteric modifiers such as F-1,6-BP have the potential although only at very high concentration to completely overcome the consequence of the phosphorylation (Blair, 1986). Until now, only insulin has been shown to activate L-type pyruvate kinase while glucagon, catecholamines (β- and α-agonists), vasopressin and angiotensin are well-known inhibitors of the enzyme (Blair, 1986; Evans, 1986; Pilkis et al., 1990). The response of liver PK is quick and reversible (Blair, 1986). Insulin can overcome glucagon inhibition of PK. Insulin favors the dephosphorylated state of the enzyme, but the exact mechanisms responsible for its action remain unclear (Blair, 1986). The complete hormonal regulation of PK is summarized in Table I. Little is known about the precise control of muscle PK activity.

d) Citric Acid Cycle

Under aerobic conditions, pyruvate will be decarboxylated to acetyl-CoA to be completely oxidized to CO_2 through a series of reactions known as the citric acid cycle or Krebs cycle. This mitochondrial cycle is unique in the sense that it is the final common pathway for the oxidation of all energy-substrates: amino acids, FA and carbohydrates. This cycle is also a source of building blocks for biosynthesis.

1° *Biochemical Pathway and Enzymes*

Pyruvate is decarboxylated to acetyl-CoA by the PDH complex. The PDH represents the point of no return for glucose carbon atoms in carbohydrate metabolism. Citrate synthase catalyzes the condensation of acetyl-CoA with OAA to form citrate which is isomerized to isocitrate via aconitase. Isocitrate is then oxidized and decarboxylated to α-ketoglutarate by isocitrate dehydrogenase, an important step in determining the overall rate of the cycle; the resulting product is further oxidized and

decarboxylated to form succinyl-CoA by the α-ketoglutarate dehydrogenase complex. The next step is the only one in the cycle that directly produced a high-energy phosphate bond in the form of GTP. Succinyl-CoA is converted by succinyl-CoA synthase to succinate which is oxidized to fumarate by succinate dehydrogenase. Fumarate is converted to malate by fumarase to finally be oxidized to OAA via malate dehydrogenase (Stryer, 1988). This cycle can give rise to twelve ATPs per molecule of pyruvate. The complete oxidation of one molecule of glucose in the muscle results in thirty-six ATPs including glycolysis, pyruvate to acetyl-CoA and the citric acid cycle (McArdle, 1991).

2° Regulatory Sites

The first regulatory control for the citric acid cycle is exerted at the irreversible reaction catalyzed by PDH complex whereby acetyl-CoA is formed from pyruvate. PDH is regulated by small molecule effectors and covalent modification (Zubay, 1988). Once past that step, glucose can not be resynthesized from acetyl-CoA. The enzyme is controlled by inhibition products, feedback regulation by nucleotides as well as by reversible phosphorylation. It is activated by AMP and inhibited by acetyl-CoA, NADH and GTP. Phosphorylation inactivates the PDH complex and it is enhanced by high ratios of ATP/ADP, acetyl-CoA/CoA, and $NADH/NAD^+$ but inhibited by pyruvate. Insulin and a high level of Ca^{2+} enhance dephosphorylation resulting in an activation of the enzyme while glucagon and β-adrenergic agonists phosphorylate and inactivate the enzyme.

The rate of the citric acid cycle is based on the need for ATP. The cycle is also controlled at the level of citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase complex. They are all regulated allosterically. ATP inhibits citrate synthase and isocitrate dehydrogenase, succinyl-CoA and NADH inhibit α-ketoglutarate dehydrogenase complex while ADP stimulates isocitrate dehydrogenase.

e) Lipogenesis

Energy storage from FA synthesis occurs only when the energy charge is high: such as when ATP/ADP and $NADPH/NADP^+$ are elevated. Contrary to glycogen stores which, during starvation, could only last for one day, human lipid stores can satisfy energy needs for as long as two months (Newsholme and Leech, 1983)

1º *Biochemical Pathway and Enzymes*

Synthesis of FA is initiated by 2-carbon atom moieties derived from acetyl-CoA. The latter is in turn derived mainly from carbohydrate metabolism and to a lesser extent amino acid metabolism (Zubay, 1988). The synthesis of FA occurs primarily in the liver and adipose tissue where they will be used in the biosynthesis of triacylglycerols. When the essential substrates are present, such as acetyl-CoA, malonyl-CoA and NADPH, synthesis of FA can proceed. The supply of acetyl-CoA and NADPH necessary for FA synthesis originated from the glycolytic pathway and the citric acid cycle. Glycolysis generates pyruvate which enters the mitochondria to react with the PDH complex leading to acetyl-CoA formation. Excess acetyl-CoA will be converted by citrate lyase to citrate which can cross the mitochondrial barrier into the cytosol where it is reconverted to acetyl-CoA by ATP-citrate synthase. In the cytosol, acetyl-CoA will react with acetyl-CoA carboxylase to form malonyl-CoA. The synthesis of FA is based on the sequential addition of 2-carbon units on a multiprotein complex.

2º *Regulatory Sites*

Acetyl-CoA carboxylase, the enzyme catalyzing the formation of malonyl-CoA from acetyl-CoA is the primary target for control. Citrate is a powerful allosteric stimulator indicating by its excess that energy needs are satisfied. Furthermore, citrate has been shown to allosterically inhibit PFK-1, thus reducing glucose uptake through an inhibition of HK due to an increase in G-6-P. These steps contribute to a putative

glucose-FFA cycle or the Randle cycle. Since its demonstration by Randle et al. (1963, 1964ab), it has never been directly demonstrated in skeletal muscle. Palmitoyl-CoA, the end product of FA synthesis is the major allosteric regulator inhibiting FFA synthesis. Acetyl-CoA carboxylase can also be controlled by covalent modification. Its phosphorylation will inactivate the enzyme. Malonyl-CoA, the first intermediate in the synthesis of FA is an important signal to avoid the simultaneous synthesis and degradation of FA (Winder et al., 1989, 1990; Elayan and Winder, 1991). Malonyl-CoA is a strong inhibitor of carnitine acyl-transferase I, the enzyme responsible for the translocation of FA from the cytosol into the mitochondrion by transferring FA from acyl-CoA to carnitine. The rate of β-oxidation is thus inhibited by the action of malonyl-CoA on the transferase, reducing the simultaneous catabolism and synthesis of FA in the liver. Glucagon and epinephrine in the liver as well as in the adipose tissue will trigger the cAMP cascade resulting in an activation of a cAMP-dependent protein kinase with phosphorylation and inactivation of acetyl-CoA carboxylase.

II. QUANTITATIVE ASPECTS OF GLUCOSE TURNOVER

A. Methodology

a) Glucose Turnover

The study of glucose turnover rates *in vivo* allows us to put in perspective the dynamic regulation of glucose metabolism under various situations. It gives more information about the metabolic situation than the simple measurement of substrate concentrations which represent only static parameters of metabolism. Two different techniques have been widely used: the arteriovenous difference and the isotopic dilution techniques.

1° *Arteriovenous Difference Techniques*

The arteriovenous difference technique is based on the arteriovenous difference of a substrate across a limb or an organ (i.e. liver) times its flow rate. Arteriovenous difference is based on the Fick equation [Blood flow x (arteriovenous)] which only gives an appraisal of the net balance of either substrate utilization or production. The major limitation of this technique is the inaccuracy in the measurement of the flow rate which could lead to an error *in vivo* of as much as 20% (Andres et al., 1956). When arteriovenous flow can be measured correctly, the results give the net balance across the limb or the organ, without specification about the absolute rate of uptake and/or release of the chosen substrate (Wolfe, 1984).

This limitation is even more important when splanchnic balance is to be measured. The blood vessels conveying blood to the liver are the hepatic artery (30% of its supply) for the oxygenated blood, but the majority (70% of its supply) of its inflow is venous blood rich in the products absorbed from the gastrointestinal tract. The blood leaves the liver through the hepatic vein directly into the inferior vena cava (Snell, 1981;

Ganong, 1983). Splanchnic balance results from the arteriovenous difference of arterial blood and hepatic venous blood in contrast to the net hepatic balance which needs the portal venous concentration and flow with the arteriohepatic venous difference (Wolfe, 1984). It should be remembered that the product of the arterial hepatic venous concentration difference of any given substrate and the splanchnic blood flow provides only an estimate of splanchnic exchange. Since the determination of the net hepatic balance requires multiple net balances across many organs such as the liver, the intestines, the pancreas, and the spleen, these are not possible by non-invasive technique in human subjects. Therefore, human studies express net splanchnic balance instead of real hepatic balance. However, a change in the net splanchnic balance could represent either a change in hepatic release or uptake, or a variation in uptake by the gut or both of them (Wolfe, 1984).

A difference exist when the product of hepatic blood flow and arteriovenous gradient is plotted against isotopic dilution values of hepatic glucose production (Ferrannini and Groop, 1989). As shown in this study, arteriovenous technique underestimated systematically when compared in the same situation and this is due to the small but significant uptake (0.2 mmol/min) of glucose by the splanchnic tissues (which occurs in both gut and liver) (DeFronzo et al., 1983; Björkman, 1986). Studies of splanchnic glucose metabolism using a combination of tracer and catheterization techniques have demonstrated that splanchnic glucose production underestimates hepatic glucose production by almost 25%. Nevertheless, the over or underestimation from either of the techniques in most situations is not critical because it is the magnitude of the difference with or without intervention, which is important in the study of metabolic regulation.

Because the arteriovenous technique requires that the hepatic vein catheter be positioned under fluoroscopic control, it is considered invasive and requires sterile

equipment. A catheter also has to be inserted into an artery which is not without complications especially during exercise.

2º *Isotopic Tracer*

The use of radioactive tracers in biomedical research is subject to very strict legal regulation. It is obvious for this reason, that glucose turnover in man has to be measured by the use of stable isotopes which are free of risk for volunteers (Reinauer et al., 1990; Jones and Leatherdale, 1991). Moreover, stable isotopes permit multiple studies in the same subject. They also can be used free of risk in children and pregnant women. Bier was a pioneer in the utilization of stable isotopes for glucose turnover measurements in man (Bier et al., 1973). Tracer technology was developed to circumvent some of the problems encountered with the arteriovenous difference technique and to enable the quantification *in vivo* of various aspects of energy metabolism (Wolfe, 1984). In this section, we shall consider only glucose turnover and gluconeogenesis methodologies.

The basic principle of tracer methodology as presented by Wolfe in his book Tracers in Metabolic Research (1984) is that "the rate of appearance of unlabeled molecules is determined by the dilution of the infused labeled molecules". One should always keep in mind the multiple subtleties inherent to the use of the isotope dilution technique (Jackson et al., 1984). Glucose turnover from isotope dilution can not provide arteriovenous differences across tissues, and thus it can not detect glucose uptake by muscle from that by other organs and tissues such as liver, brain and erythrocytes (Jackson et al., 1984).

For our purposes, an augmentation in muscle glucose uptake induced by exercise could be balanced by a reduction in glucose uptake by other organs or tissues due, at least in part, to the reduced delivery of glucose to those tissues. Glucose

production (*Ra*) and glucose utilization (*Rd*) when measured by tracer dilution reflecting whole body glucose kinetics during mild to moderate intensity exercise do not exactly reflect what is going on at the level of the specific exercising muscle (Shilo et al., 1990).

Studies were done with the use of a number of glucose tracers labeled with either radioactive or stable carbon or hydrogen isotopes. Administration of the chosen tracer is done by single injection or by prime constant infusion (Ferrannini et al., 1985). Glucose tracers labeled on position 2 with hydrogen slightly overestimate glucose production because in this position, the labeled carbon is lost in the conversion of G-6-P to F-6-P (Dunn et al., 1976). ^{13}C-labeled glucose overestimated the rate of glucose production from non-recycled glucose carbons (Wolfe, 1984). Deuterated glucose with deuterium on positions 2, 3, and 6 are utilized most often and the deuterium is lost at different stages of glycolysis and gluconeogenesis. Glucose molecule labeled on position 2 is lost in the hexose-isomerase reaction (G-6-P to F-6-P), while the one labeled on position 3 is exchanged with protons and water at the level of the triose phosphate isomerase where DHAP is isomerized to glyceraldehyde 3-phosphate. Labeled atoms on position 6 are lost at the PC reaction and during equilibration between OAA, malate and fumarate in the equilibration of the mitochondrion hydrogen pool (Wolfe, 1984).

Hepatic glucose production, however, derived by any tracer dilution technique, is a good estimate of the authentic rate of free glucose from the G-6-P reaction (Ferrannini and Groop, 1989).

b) Gluconeogenesis

Gluconeogenic rate can be estimated by measuring the incorporation of isotopically labeled gluconeogenic precursors into plasma glucose or by measuring net splanchnic uptake of gluconeogenic substrates. Both methods carry their own limitations and they have to rely on multiple assumptions.

1° *Arteriovenous Difference Techniques*

Using the hepatic vein catheter technique described previously, splanchnic uptake of gluconeogenic precursors can be determined. This provides an estimate of the rate of hepatic gluconeogenesis and allows one to determine the relative contributions of gluconeogenesis versus glycogenolysis to the total splanchnic glucose production. As explained earlier, however, the arteriovenous technique isolates the splanchnic bed instead of the liver. Depending on the nutritional state, the gut can release alanine and lactate into the portal circulation. Under these conditions, the arteriovenous difference across the splanchnic bed will underestimate the actual hepatic uptake of gluconeogenic precursors. This technique only yields gluconeogenic precursors extraction by the splanchnic bed not their respective incorporation into glucose (Chiasson et al., 1977). Effectively, alanine and lactate can enter many metabolic pathways only one of which being gluconeogenesis. This estimation thus represents the maximal rate of gluconeogenesis if all of the gluconeogenic precursors taken up by the hepatic tissue were converted to glucose (Björkman, 1986).

2° *Arteriovenous Difference Techniques and Isotopic Tracer*

A combination can be done with a labeled gluconeogenic precursor, [^{14}C]-alanine for example, and the arteriovenous hepatic vein difference technique. With this approach a catheter is placed in an hepatic vein and another one in a peripheral artery. This method gives a direct measurement of the splanchnic extraction of the gluconeogenic precursors and the release of [^{14}C]-glucose by the liver. Conversion of alanine to glucose can then be assessed from hepatic flow (measured by indocyanin green), and by dividing the production of [^{14}C]-glucose by the specific activity of [^{14}C]-alanine (from the hepatic vein preferably). This method remains invasive because of the requirement for hepatic vein catheterization (Chiasson et al., 1977).

3° *Double Tracer Methodology*

Labeled gluconeogenic precursors, ^{14}C or ^{13}C, can be utilized in order to trace their conversion into glucose. Total glucose pool can also simultaneously be determined from a different tracer labeled with either tritium or deuterium. These *in vivo* techniques have limitations; the most critical being the loss of labeled carbons at the level of the OAA pool (Hetenyi, 1982; Wolfe, 1984; Kelleher, 1986). Other factors could also influence the estimation of gluconeogenesis, such as the equilibration of OAA with fumarate, futile cycling and the sampling site for precursors (Brosnan, 1982; Katz, 1982; Wolfe, 1984; Cobelli and Ruggeri, 1989; Wisneski et al. 1990).

OAA stands at a crossroad where it is either formed from acetyl-CoA oxidation in the Krebs cycle or from lactate, alanine or other amino acids through pyruvate. This results in a mixture of the labeled precursors, such as [^{14}C]-lactate or [^{14}C]-alanine, with the unlabeled OAA coming from the Krebs cycle leading to an underestimation of gluconeogenesis by a dilution of the specific activity of the labeled carbons (Hetenyi, 1982; Wolfe, 1984). OAA has to be converted into malate and then into fumarate for transportation across the mitochondrial barrier. If the activities of the enzymes responsible for these two steps, malate dehydrogenase and fumarase are increased *in vivo* more than the Krebs cycle, OAA will be transformed into malate, and then into fumarate before transportation from the mitochondrion into the cytoplasm for *de novo* glucose synthesis. If only malate was involved, the labeled carboxyl could be lost because it could be removed by PEPCK (Fig. 6). However, if all malate is transformed in fumarate, one out of every two carbons could be retrieved as glucose. *In vivo*, there is an equilibration between malate and fumarate concentration, so it probably interferes with the estimation of gluconeogenesis (Brosnan, 1982).

Blood sampling site is also an important issue in the measurement of gluconeogenesis (Katz, 1982). Theoretically, one would like to be able to measure the specific activity of the gluconeogenic precursor exactly as it is taken up by the liver. In man, this is not feasible because 70% of the circulation reaching the liver comes from the portal vein and only 30% comes from the hepatic artery. So the next best site would be an arterial site since the precursors will reach the liver through both the hepatic artery (30%), and the splanchnic arteries (70%) by way of the portal vein. But even arterial catheterization is considered invasive and is not without complications. This problem can be overcome by using "arterialized" venous blood. In order to do so, samples are drawn from a hand vein maintained at 68° centigrade by warming up the hand to induce an increase in blood flow and vasodilation, resulting in arteriovenous shunts (Abumrad et al., 1981). With this technique, venous blood samples have the same composition as arterial blood which is more representative of the whole body response than the exact location of the catheter (Wolfe, 1984).

Chiasson et al. (1977) validated a technique that uses two radioactive isotopes and that evaluates simultaneously glucose turnover and gluconeogenesis by comparing it with the arteriovenous catheterization technique combined with a radioactive tracer. More recently, Lecavalier et al. (1985) extended this approach to stable isotopes and validated the technique against the reference method using radioactive tracers. The tracers used are D-[2,3,4,6,6-$^{2}H_5$]-glucose and L-[1,2,3-^{13}C]-alanine. The specific samples activity of ^{13}C-alanine is measured from an arterialized hand vein. This results in a slightly higher specific activity than what the liver sees because of the dilution normally occurring in the splanchnic bed in the postabsorptive state. This results in a slight underestimation of the calculated conversion of alanine to glucose. The specific activity of the precursor is further diluted in the oxaloacetatate pool, thus increasing the underestimation of net gluconeogenic rate. For this reason, our technique provides us

with only an index of gluconeogenesis rather than a quantitative measurement of the gluconeogenic rate. This still provides us with a tool to study changes in the rate of gluconeogenesis under various conditions, and therefore, is still valid to study the regulation of this metabolic process. Cherrington and colleagues have shown that the precursor dilution due to a change in proteolysis or glycolysis in a dynamic situation, is minimal when compared to the changes in gluconeogenic rate (Cherrington et al., 1977; Stevenson et al. 1987).

A factor has been proposed by Hetenyi to correct for the precursor dilution at the level of the OAA pool (Hetenyi, 1982). The factor of Hetenyi has been calculated to be 2.2 and since no difference has been found even with drastic hormonal perturbations, it is the correction factor in most cases (Hetenyi, 1982). These limitations of the technique to measure gluconeogenesis *in vivo* have been discussed in a number of studies (Katz et al., 1974; Katz, 1985; Kelleher 1986; Katz and Grunnet, 1979).

Even with these limitations, this technique provides a qualitative index of gluconeogenesis which allows the study of hormonal regulation of this metabolic process in healthy or diabetic subjects at rest, during a meal, and even during exercise (Chiasson et al., 1977; Martineau et al. 1985).

B. Glucose Turnover in the Postabsortive State

a) Hepatic Glucose Production

After a 12-hour overnight fast, when no carbohydrates are delivered into the circulation from the intestinal tract, the liver is the sole supplier of glucose. Under these conditions, it has been demonstrated that 75% of the circulating glucose is derived from glycogenolysis and the remaining 25% from gluconeogenesis (Wahren et al. 1971, 1978). The overall glucose utilization by the resting skeletal muscle is around 20 to 25 mg per minute, representing less than 10-15% of the total body glucose turnover (Felig and

Wahren, 1975). Most of the glucose taken up in the postabsorptive resting state is probably directed to storage in the form of glycogen and/or converted to lactate or pyruvate since the respiratory quotient in the resting state is about 0.7, showing a total energy dependence of muscle on FA oxidation (Andres et al., 1956; Wahren et al, 1975). Muscle is therefore not an important user of glucose in the resting state, the major glucose consumption site being the brain (Wahren, 1979).

1° *Total Ra*

In the resting state, hepatic glucose production equals more or less 150 mg/min or 833 µmol/min. The liver represents 2.5% of the body weight and contains normally 50 g of glycogen/kg while the normal muscular glycogen reserve is more or less 15 g of glycogen per kg of muscle (Péronnet et al., 1983). The average glucose consumption of the brain is around 4 g of glucose per hour or 67 mg/min, thus the importance of the liver in providing a continuous supply of glucose between meals can not be underestimated. Hypoglycemia is prevented by the regular release of glucose from the liver into the circulation (Gollnick, 1988).

Table III represents data from studies in overnight fasted humans. Hepatic glucose production is expressed in $\mu mol \cdot kg^{-1} \cdot min^{-1}$. From this table, we can see that estimates of postabsorptive hepatic glucose production ranged from 8.16 to 18.92 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ when using the arteriovenous difference technique, from 10.3 to 13.2 when using radioactive tracers and, finally from 7.3 to 13.4 when using stable isotope tracers (Cahill and Owen, 1968; Ahlborg et al., 1974; Wahren et al., 1975, 1978; Rizza et al., 1979, 1980; Saccà et al., 1979 Wahren, 1979; Björkman et al., 1981; Tserng and Kalhan, 1983; Jacksson et al., 1984; Jenkins et al., 1985; Martineau et al., 1985; Cobelli et al., 1987; Consoli et al., 1987; Knapick et al., 1988; Lecavalier et al., 1989, 1990; Levy et al., 1989; McMahon et al., 1989; Consoli and Nurjhan, 1990; Reinauer et al., 1990;

Vikamäki et al., 1990; Clore et al., 1991). From these data, we can estimate that hepatic glucose production in the postabsortive state to be 11.39 ± 0.29 µmol•kg^{-1}•min^{-1} when the twenty-seven values are averaged with the exclusion of the lowest and the highest values. In fact, the various techniques give similar mean results: 11.23 ± 0.74 µmol•kg^{-1}• min^{-1} for the arteriovenous technique, 11.68 ± 0.25 µmol•kg^{-1}•min^{-1} for the radioactive tracers, and 10.48 ± 0.88 µmol•kg^{-1}•min^{-1} for the stable isotope tracers.

Knowing that after an overnight fast, hepatic glucose production is derived in a proportion of 75% from glycogenolysis and 25% from gluconeogenesis, let us now consider some of the studies in which a gluconeogenic index was used to measure this process.

2º *Gluconeogenesis*

Gluconeogenesis comes into play as glycogen reserves decrease (Cahill, 1986). The liver carries an intrinsic feature which allows the hepatocyte to switch from a glucose storage to a glucose production organ as we pass from the fed to the fasting state or vice versa. Because of this capacity to store or release glucose, the liver is able to provide a continuous and appropriate supply of glucose for the maintenance of normoglycemia in the intervals between feedings (Saccà, 1987). Blood glucose is normally maintained between 4.5 - 6.7 mmol/L despite constant change in glucose absorption and peripheral utilization. Furthermore, to conserve glucose for the brain, the rest of the body will burn fat preferentially whenever possible (Andres et al., 1956; Halperin and Rolleston, 1990).

Table IV contains gluconeogenesis data from overnight fasted humans studies. Using the balance of gluconeogenic substrates across the splanchnic vascular bed, Wahren reported a gluconeogenic estimation of 2.55 µmol•kg^{-1}•min^{-1} when expressed as glucose equivalents and that value accounted for 31% of the total glucose

production (Wahren et al., 1975). In a similar experiment, Ahlborg and colleagues (1974) noted a contribution of gluconeogenesis to glucose production of 25% which was equal to 2.70 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Recently, Consoli et al. (1987, 1990), using a new combination of tracers, [6-^{3}H] glucose and [2-^{14}C]-acetate, to measure Krebs cycle carbon exchange, estimated gluconeogenesis in overnight-fasted healthy subjects to be around 28% (3.57 ± 0.38 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) of the overall glucose production.

Using D_5-glucose and ^{13}C-alanine, Martineau et al. (1985) reported the lowest values in resting healthy subjects after a 12-hour fast as being 0.42 and 0.51 ± 0.035 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for gluconeogenesis. These results are in agreement with those previously reported by Chiasson et al. (1979), Foster et al. (1980), and Hetenyi et al. (1983). In these studies, however, the data was not corrected for isotopic dilution in the OAA pool. Nevertheless, the technique can still be used to study changes in the metabolic process as a tool to characterize the regulation of gluconeogenesis.

Recently, a new tool has been applied in an attempt to surmount the previous bottleneck in the quest of proper quantification of hepatic gluconeogenesis and glycogenolysis contribution to hepatic glucose production. These two processes have been quantified *in vivo* in fasting subjects using ^{13}C nuclear magnetic resonance (NMR) spectroscopy in combination with [6-^{3}H]-glucose turnover. Rothman et al. (1991) indicate that the contri[illegible] gluconeogenesis to total net glucose production is more important than previously thought accounting for nearly 50% (7.9 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) of overall glucose production during the initial four to twenty-two hours of fasting.

b) Glucose Utilization

At rest, only 10% of substrate oxidation in skeletal muscle originates from glucose oxidation while FA are the predominant substrates (85-90%) with a small contribution from the amino acids (1-2%) (Ahlborg et al., 1974). FFA are thus an

important fuel for resting muscle. Recently, Kelley et al. (1990) reconfirmed that lipid was the primary oxidative fuel in the postabsorptive period for resting muscle with less than 20% of the energy substrate derived from glucose oxidation.

In the postabsorptive state, *Ra* equals *Rd*. If we considered the hepatic glucose production listed in Table III, we can estimate from these studies that glucose utilization after an overnight fast reaches 11.39 ± 0.29 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and that nearly 75% of this glucose will be utilized by non-insulin-dependent tissues.

One can also calculate the amount of glucose which is taken up by the tissues and which is actively transported across the plasma membrane independent of the mass effect: this is called the glucose metabolic clearance rate. It is calculated by simply dividing *Rd* by blood glucose concentration thus correcting for the mass effect. Reinauer et al. (1990) reported a glucose metabolic clearance rate of 3.04 ± 0.17 ml/kg•min while Lecavalier (1987) found a lower rate of 1.53 ± 0.048 ml/kg•min in postabsorptive healthy subjects. Rizza et al. (1979) reported in between rates of glucose clearance of 2.1 ± 0.2 ml/kg•min. In overnight fasted healthy subjects, Jenkins (1985), Rizza (1980) and Saccà (1979) found respectively glucose clearance rates of 2.72 ± 0.02 ml/kg•min, 2.0 ± 0.1 ml/kg•min and 2.25 ± 0.15 ml/kg•min.

c) Different Species

Though the list is far from being completed, Table V listed the results from some of dog studies. All studies were done with radioactive tracers. Using [3-^{3}H]-glucose, Stevenson et al. (1991) observed a basal endogenous hepatic glucose production of 13.44 ± 2.67 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and a glucose metabolic clearance of 2.42 ± 0.32 ml/kg•min. Saccà (1978, 1979a) reported a basal glucose turnover of 11.22 ± 1.39 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ after a 16 to 18-hour overnight fast in dogs using [3-^{3}H]-glucose. Glucose turnover in overnight fasted female dogs (19 to 29 kg) reached 15.39 ± 1.05 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ using

[3-^{3}H]-glucose in Myers' study (1991). Radziuk (1978) noted variations in glucose turnover from 13.55 to 20.55 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in dogs with either [6-^{3}H]-glucose or [1-^{14}C]-glucose (1978).

Other investigators with dog studies using radioactive tracers have observed similar results (Altzuler et al., 1968, 1976; Cowan and Hetenyi, 1971; Vranic et al., 1976b; Cherrington et al., 1977, 1984; Rizza et al., 1981; Wolfe and Shaw, 1986; Ader and Bergman, 1990). It can be seen from Tables III and V that hepatic glucose production is slightly greater in dogs compared to human subjects.

C. Glucose Turnover During Exercise

a) Introduction

1° *Metabolic Adaptation to Physical Exercise*

Exercise requires major cardiovascular and metabolic adjustments to meet the increasing need for oxygen and energy-substrates for the working muscles while maintaining sufficient oxygen and fuel supply to the brain and other vital organs (Maughan, 1992). With increasing duration and intensity of exercise, the contracting muscle has the unique ability to use a wide variety of substrates as fuel.

The main energy sources for exercising skeletal muscles are glucose and FFA. The relative contribution of these fuels and the metabolic response depend on several variables: duration and intensity of exercise, degree of cardiorespiratory fitness, age, sex, nutritional and endocrine status (Galbo 1983, 1992). After an overnight fast, the major fuel for the resting muscle is FFA derived from adipose tissue (Ahlborg et al., 1974) while glucose has only a minor role. With the onset of exercise, the utilization of carbohydrates as fuel increases in direct proportion to the intensity of the exercise (Wahren, 1979; Richter and Galbo, 1986). As reviewed by Cooper et al. (1989), the

higher intensity of exercise, the higher is the glucose uptake. In addition to an increase with greater exercise intensity, the uptake of blood glucose also decreases as the duration of exercise increases (Coggan and Coyle, 1991). Muscle glycogen is utilized during the first few minutes concomitantly with CP (Gollnick, 1988). Carbohydrates will be supplied by both the increase in glucose uptake and the glycogen breakdown by and within the muscle cell, respectively. The increase in glucose uptake by contracting muscle is due to both an increase in the Vmax of glucose transport and a decrease in the half saturating substrate concentration (Km) (Douen et al., 1989; King et al. 1989; Goodyear et al., 1990; Klip and Pâquet, 1990). It is now known that muscle contractions *per se* induce the translocation of the glucose transporters (GLUT4) from the cytoplasm to the plasma membrane (Sternlicht et al., 1989; Klip and Pâquet 1990; King et al 1989; Goodyear et al 1990; Douen et al 1989; Young et al., 1991), resulting in a transfer of glucose across the plasma membrane in the direction of the glucose gradient. The glucose taken up by the muscle and that supplied by the breakdown of glycogen will increase the flux of glycosyl units through the glycolytic pathway for the conversion to pyruvate and acetyl-CoA, oxidation in the citric acid cycle and ATP production (Richter et al., 1981). The key rate-limiting enzymes involved here include GPhosp, PFK-1 and PDH (Newsholme and Leech, 1983).

2º *Duration and Intensity*

Duration. If exercise is prolonged, however, plasma FFA will again become the major energy yielding substrate for the working muscle (Gollnick and Saltin, 1988). In fact, during prolonged exercise of mild to moderate intensity (40% VO_2 max), FFA oxidation accounts for approximately 60% of muscle oxygen consumption (Ahlborg et al., 1974). The uptake of FFA by muscle is not hormone-dependent and at a given workload, it is generally proportional to the concentration of plasma FFA (Hagenfeldt, 1979; Holloszy, 1990). However, it has recently been shown that FFA uptake follows

saturation kinetics (Turcotte et al., 1991) and that a fatty acid binding protein in the plasma membrane of skeletal muscle might be involved in the translocation process (Turcotte et al., 1992). Furthermore, high FFA levels will have a sparing effect on glucose utilization by interfering with glucose uptake and glycogen breakdown through the glucose-fatty acid cycle originally described by Randle in the heart (Randle et al., 1964) and by Rennie and Holloszy (1977) in red skeletal muscle. This cycle works via an inhibition of PFK-1 by citrate with an accumulation of G-6-P and free glucose (Randle et al., 1964; Hargreaves et al., 1991). Björkman and colleagues (1988) reported that in dogs running for 30 to 60 minutes glucose metabolic clearance rate decreases as plasma FFA concentration increases. FFA never fully replaces the need for glucose and even during prolonged exercise, blood glucose constitutes an important substrate for contracting muscle (Ahlborg et al., 1974). As a matter of fact, glycogen depletion has been shown to coincide with exhaustion (Hermansen et al., 1967). However, it has been reviewed recently by Green (1991) that fatigue might also be cause by other non-metabolic factors.

To prevent hypoglycemia during exercise, the increase in glucose utilization by working muscles must be counterbalanced by an increase in hepatic glucose output (Wahren et al., 1971; Wahren, 1979). During exercise, the liver is the main organ responsible for the release of glucose into the circulation (Wahren et al., 1971). In the postabsorptive state, the increase in hepatic glucose production at the beginning of exercise is mainly due to glycogenolysis. As exercise continues in the absence of carbohydrate intake, gluconeogenesis plays an increasingly important role (Ahlborg et al., 1974; Turcotte et al., 1990). The principal gluconeogenic precursors during prolonged exercise are lactate, alanine and glycerol (Felig and Wahren, 1971; Ahlborg et al., 1974). Thus, during exercise, normoglycemia is maintained because the sum of the exercise-induced augmentation in hepatic glycogenolysis and gluconeogenesis meets the increased rate of muscle glucose utilization (Wasserman and Cherrington, 1991).

Intensity. On the other hand, as the intensity of exercise increases, glucose progressively becomes a more important substrate for energy production. The rate of blood glucose uptake during exercise is curvilinearly related to the intensity of exercise (Wahren et al., 1971; Wahren, 1979; Katz et al., 1986; Richter and Galbo, 1986). When working at 50% VO_2 max, muscle derives approximately 50% of its energy from glucose oxidation. At intensities of 70 to 75% VO_2 max, glucose becomes the predominant metabolic fuel and when energy is at near 100% VO_2 max, most of the energy is derived from glucose oxidation. The high rate of glycogen consumption limits the duration of muscular activity (Pernow and Saltin, 1971; Sjodin, 1992). The accumulation of anaerobic metabolites or the depletion of energy reserves may be directly or indirectly responsible for the impairment of muscular function (Sahlin, 1986; MacLaren et al., 1989; Hultman et al., 1990). Amino acids contribute only 1-2% of the energy requirement during muscle contractions at all intensities of exercise and oxidation of FFA makes up the difference (Felig and Wahren, 1975). Thus, at very high intensity of exercise, glucose oxidation rates are markedly increased, muscle glycogen is rapidly depleted and glucose uptake from the circulation is high. If hepatic glycogen stores are adequate, hepatic glucose production is able to match or even exceed peripheral utilization and blood glucose concentration remains constant or may actually increase (Horton, 1988; Marliss et al., 1991).

At all working intensities, increasing the duration of exercise results in a further fall in muscle and hepatic glycogen and in an increase in plasma FFA concentration. FA oxidation by exercising muscle increases gradually and glucose oxidation decreases. Hepatic glucose production decreases and becomes more dependent on gluconeogenesis which is usually sufficient to maintain normal blood glucose concentration (Horton, 1988; Turcotte et al., 1990). Exercise stimulates three of the steps involved in the stimulation of gluconeogenesis such as gluconeogenic precursor delivery

to the liver, extraction by the liver, and efficiency of conversion to glucose within the liver. Accelerated rates of proteolysis, lipolysis and glycolysis increase amino acids, glycerol, lactate and pyruvate production and may enhance their hepatic availability (Wasserman and Cherrington, 1991).

An orderly sequence of fuel utilization during light to moderate exercise for prolonged bouts may be viewed as a triphasic sequence where intramuscular substrates, notably glycogen, blood glucose, and FFA are successively of paramount importance as the major energy-yielding fuel (Wahren, 1979). Intramuscular substrate stores such as CP and glycogen seem more important at the start of work and during high intensity work while circulating FFA and glucose are the predominant fuels during prolonged work (Björkman, 1986; Björkman and Wahren, 1988; Gollnick and Saltin, 1988).

a) Hepatic Glucose Production

1° *Total Hepatic Glucose Production*

Independently of the technique utilized by different groups, exercise increases *Ra* as well as *Rd* and the magnitude of the increments depends on the intensity and duration of the chosen exercise. When looking at the published exercise studies, glucose production undeniably increases during exercise (Wahren et al., 1971, 1975; Ahlborg et al, 1974; Sestoft et al., 1977; Wahren, 1977; DeFronzo et al. 1981; Ahlborg and Felig, 1982; Jenkins et al., 1985, 1986; Knapik et al., 1988; Stanley et al., 1988; Cooper et al., 1989; Shilo et al., 1990; Weber et al., 1990; Wasserman et al., 1991b). Similar results have been obtained whether the isotopic dilution technique or arteriovenous difference technique has been used. Interestingly, both techniques showed that the duration of the exercise seems to be of minor importance for the magnitude of the increase in glucose production during exercise (Table VI).

As a matter of fact, when you plot the increase in hepatic glucose production against the intensity of exercise for a duration of 30 to 60 minutes, we can clearly see that hepatic glucose production increases with exercise intensity with a correlation of 0.75 (Fig. 7). However, the increase in hepatic glucose production is less manifest when it is plotted against the duration of exercise at an intensity of 40 to 60% VO_2 max (Fig. 7).

2° *Gluconeogenesis*

Table VII lists the human studies in which gluconeogenesis was estimated. Again, a wide distribution was observed. As expected, high intensity exercise was less dependent on gluconeogenesis for glucose production. This is in agreement with the primary use of hepatic glycogen as a source for glucose production in the first minutes of exercise to account for hepatic glucose production and as the intensity of exercise is increased until the glycogen is depleted. On the other hand, low intensity exercise (around 30% VO_2 max) of a longer duration is more dependent on gluconeogenesis as estimated from the precursors balances across the splanchnic bed. A study using ^{14}C-lactate showed a contribution of lactate to gluconeogenesis of 25% when exercising at 43% VO_2 max for 30 to 50 minutes and it appeared that direct oxidation of lactate primarily in exercising skeletal muscles and the myocardium was predominant over its reuptake for glucose formation during submaximal exercise (Stanley et al., 1988). In rats, Turcotte and colleagues (1990) showed the importance of gluconeogenesis for the maintenance of blood glucose homeostasis by inhibiting PEPCK in short-fasted running rats.

b) Glucose Utilization

Increased glucose uptake by contracting muscles has long been recognized (Chauveau and Kaufmann, 1907). The increase is much more important when one

consider glucose uptake by the exercising leg instead of whole body glucose uptake (Table VIII). As noted by Cooper et al. (1989), an increase in muscle glucose uptake during exercise is usually matched by a reduction in glucose uptake by other tissues, due to a reduced delivery of glucose to those tissues secondary to a decrease in blood flow. This is why when one compared the leg glucose uptake using the catheterization technique, the magnitude of the increase in glucose uptake was enormous (7- to 23-fold) compared to whole body glucose kinetics using the isotopic dilution technique (1.1- to 2.5-fold).

In Figure 8, exercise glucose uptake is plotted against intensity (A) and duration (B) of exercise. In order to be able to consider together in the same graph leg glucose uptake with whole body glucose uptake, we rearranged the values as follow: knowing that mean resting leg glucose uptake in Wahren's study (Wahren et al., 1971) equaled 0.178 mmol/min and resting glucose utilization (*Rd*) was around 0.865 mmol/min. By dividing the latter from the former, we obtained a factor of 4.86. We used this factor in order to convert leg glucose uptake into a predicted estimation of whole body glucose uptake. In Figure 8A, we can see that for an exercise duration between 30 to 60 minutes, glucose uptake increases with the intensity of exercise. This is in agreement with data found in the literature. In Figure 8B, when glucose uptake during exercise at 40 to 60% VO_2 max was considered against duration of exercise in minutes, the dependence was less obvious. The relationship between the increase in glucose uptake and the duration of exercise only reached an r of 0.2. This failure to show a direct relationship between glucose uptake and duration of exercise is probably due to the increase in FFA utilization over glucose utilization as duration of exercise is prolonged.

In the few studies where glucose metabolic clearance was calculated, the result ranged from no increase at all to a 2-fold increase over basal glucose metabolic clearance

(Table IX) (Jenkins et al., 1985; Stanley et al., 1988; Cooper et al., 1989; Kjær et al., 1990).

c) Glucose Turnover From Arteriovenous Differences And Isotopic Studies in Dogs

Dog studies used treadmill running with or without a 10 to 15% slope. The speed ranged from 100 to 133 m/min and the duration of the exercise from 65 up to 240 minutes. Most of the dog studies reported in this section used the isotopic dilution technique with one or two radioactive tracers mainly [3-^{3}H]-glucose, [U-^{14}C]-glucose, and [U-^{14}C]-alanine coupled to indocyanine green dye for the determination of hepatic blood flow. Only studies done in the postabsorptive state will be discussed. Issekutz (1967, 1979, 1980, 1981) found an increase of 2.5 to 2.75 in *Ra* and *Rd* with a glucose metabolic clearance rate augmented by exercise from 2.9 to 3.6 times. Vranic et al. (1969, 1979, 1984, 1976a, 1988) observed an enhancement of *Ra*, *Rd* and glucose metabolic clearance rate of 1.5, 1.7, 1.8, respectively during horizontal running. When a slope of 10 to 12% was added, a higher value of 2.8 was observed for *Ra* and *Rd*. Wasserman et al. (1984, 1985, 1988, 1989abcd, 1990, 1991c) found a similar increase in *Ra* (1.5 x), *Rd* (2.9 x) and glucose metabolic clearance (1.8 x) during moderate intensity exercise at 100 m/min at 12% grade for 90 to 150 minutes. In some of Wasserman's studies (1989cd), they measured the conversion of alanine to glucose, and observed a 212 to 248% increase over baseline by the end of exercise. In the same studies, they also reported a 3.3-fold increase in hepatic uptake of precursors over resting value with a gluconeogenic efficiency 5.4-fold higher at the end of the 150-minute exercise period (Wasserman et al., 1990). From these data, it can be calculated that 37% of total *Ra* was derived from gluconeogenesis.

Taken together, the studies on glucose turnover and gluconeogenesis after an overnight fast and during exercise clearly emphasize the importance of gluconeogensis in the contribution to total glucose production. Its relative importance during exercise will depend on the intensity and duration of the chosen activity. This fine regulation is dependent, at least in part, on the hormonal environment.

III. HORMONAL REGULATION

A. Potential Hormones And Their Regulatory Sites

Glucose homeostasis is finely regulated on a minute to minute basis by complex hormonal interactions. Insulin, glucagon, catecholamines, cortisol and growth hormone are the most important hormones known to be involved in glucose metabolism particularly during exercise.

a) Insulin

1° *Source*

It is well known that insulin is secreted by the endocrine pancreas, more precisely by the β cells of the islets of Langerhans. Insulin is a peptide hormone of fifty-one amino acids and is composed of two chains, the A and B chains, linked by disulfate bonds (Girard and Ferré, 1990). Insulin secretion is under the control of glucose, amino acids and the autonomic nervous system. Recently Bobbioni-Harsch et Jeanrenaud (1990) showed that a hypothalamic peptide could act as a releasing hormone that can directly influence insulin secretion. Exercise as well as training will modulate insulin secretion in response to glucose (Galbo, 1983, 1992).

2° *Insulin Receptor*

Several decades after the discovery of insulin by Banting and Best, the mechanism of insulin action still remains one of the mysteries of cell biology. Insulin has been extensively investigated, because insulin related pathologies are life threatening (Unger and Foster, 1992).

It is well known that insulin action on target cells generates a complex response that affects carbohydrates, lipids and protein metabolism. Insulin's anabolic

effects are referred to as either metabolic or growth promoting (Czech et al., 1988; Unger and Foster, 1992). Insulin first interacts with specific receptors on the plasma membrane of target tissues (Sibley et al., 1988).

Although, exact transmembrane signaling mechanisms have yet to be resolved, intracellular changes occur within few minutes after insulin binding (Rosen, 1987). These changes include modifications in the state of key controlling enzymes either through phosphorylation (Larner, 1971) or dephosphorylation (Brownsy and Denton, 1982), and that lead to inhibition of lipolysis, stimulation of protein biosynthesis and of lipogenesis, promotion of cell growth, redistribution and translocation of certain proteins such as the insulin-sensitive glucose transporter GLUT4 (Denton, 1987; Bell et al., 1990) and stimulation or suppression of gene expression (Rosen, 1983, 1987). All of these actions are mediated by a specific high affinity receptor.

The insulin receptor is a transmembrane glycoprotein, which recycles between the plasma membrane and an intracellular pool (Rosen, 1989; Taylor, 1991). It is composed of two different pairs of subunits: two α-subunits (M_r 135 000) which are linked by disulphide bonds, and two β-subunits (M_r 95 000). The insulin receptor is a protein tyrosine kinase receptor as shown in Figure 9.

Two important features of the insulin receptor deserve to be mentioned. First, although both subunits are exposed to the extracellular environment only the β-subunit spans the membrane and is exposed to the intracellular milieu. Second, the β-subunits exhibit a specific enzyme, tyrosine kinase activity, that phosphorylates proteins on their tyrosine residues rather than on serines and threonines which are phosphorylated by the cAMP-dependent protein kinase (Mendelson, 1988; Rosen, 1989).

The α-subunit contains the insulin binding site while the β-subunit is the transducing element which transduces insulin information (Goldfine, 1987). The ligand

insulin is internalized with its receptor and insulin is passed to lysosomes allowing its degradation while the receptor is recycled back to the cell membrane (Taylor, 1991).

The α-subunit of the insulin receptor seems to exert an inhibitory effect to limit the tyrosine kinase activity of the β-subunit. Once insulin is bound, it stimulates autophosphorylation of the receptor and other proteins by tyrosine kinase (Rosen, 1989). Insulin binding is necessary only for the initiation of tyrosine kinase activity, and as tyrosine kinase activity is maintained by autophosphorylation, insulin binding is no longer required (Rosen, 1983). This autophosphorylation of the β-subunit activates the kinase activity toward other substrates (Rosen, 1987, 1989).

When looking at the effects of insulin on key enzymes, it seems paradoxical that insulin could mediate both phosphorylation as well as dephosphorylation. This apparent paradox found its answer in the discovery of two distinct pathways: 1) insulin signaling involves a phosphorylation cascade initiated from the tyrosine kinase activity of the receptor (Larner, 1979); and, 2) the generation of a second messenger, acting analogous to cyclic nucleotides or inositol phosphates (Kasuga, 1982). A novel glycosylated inositol by-product produced by the hydrolysis of a membrane-linked phosphoinositol is involved (Saltiel, 1990). These two pathways may not be mutually exclusive (Czech et al., 1988). In fact, they may work synergistically to produce insulin cellular responses. In the liver, it seems that insulin decreases cAMP concentrations through an increase in phosphodiesterase activity, but inhibition of the cAMP-dependent protein kinase might be responsible for insulin's effect on hepatic glucose production (Gabbay and Lardy, 1984).

The amplification of the insulin signal arises from the phosphorylation cascade from tyrosine kinase. Some hundreds of receptors occupied by insulin bring activity to approximately 10^5 molecules of target enzyme in the cell. It is suggested by

Taylor in his excellent review of insulin action that the amplification cascade implicates two steps: 1) receptor tyrosine kinase phosphorylating and activating a serine kinase; and 2) activated serine kinase phosphorylating enzymes, which dephosphorylate regulatory enzymes on serine or threonine moieties (Taylor, 1991; Czech et al., 1988).

3º *Target Cells and Enzymes*

Our attention will be focused only on the action of insulin on glucose transporters and the activation of major target enzymes while insulin action on gene expression will be left aside. Insulin action can be observed in the liver as well as on peripheral tissues such as muscle and adipose tissue. *In vitro* hepatic insulin action is only demonstrable following prior glucagon activation. Thus in hepatocytes, insulin is considered to be a counterregulatory hormone to glucagon. However, in non-hepatic tissues, direct effects of insulin can predominate, thus conveying a regulatory role to insulin (Griffin, 1988).

4º *Glucose Transport*

One of the best known response of insulin administration is the acute activation of transport of glucose from plasma across the cell membranes. Glucose transport into muscle cells and adipocytes is rate-limiting for carbohydrate metabolism under physiological conditions of insulin stimulation (Yki-Jarvinen et al., 1987, 1991). Glucose transporters are facilitated diffusion channels and a specific insulin-dependent transporter (GLUT4) exists in muscle and adipose tissue (James et al., 1989; Bell et al., 1990; Kasanicki and Pilch, 1990; Klip and Pâquet 1990; Thorens et al., 1990). Cellular redistribution of glucose transporters has been detected in response to acute exposure of muscle to insulin (Klip et al., 1986; Klip and Pâquet, 1990). In muscle cells and adipocytes, glucose transport rate increases 20- to 40-fold in response to insulin (Kasanicki and Pilch, 1990). Insulin action mediates translocation of this transporter

from an intracellular pool to the cell membrane (Cushman and Wardzala, 1980). An increase in the number of glucose transporters in the plasma membrane and decrease in the number of transporters associated with internal membranes from rat muscles have been shown after insulin injection (Klip et al., 1986; Klip and Pâquet, 1990). This, however, does not bring a change in the number of glucose transporters suggesting that synthesis of transporters is not involved in the acute insulin response (Klip and Pâquet, 1990). The effects of insulin could be mediated via activation of a protein kinase C isoform by diacylglycerol which activates glucose transport (Girard and Ferré, 1990; Ishizuka et al., 1990). The signaling chain linking the insulin receptor to the transport of glucose is not yet fully understood. It has been proposed that a phosphorylation/dephosphorylation cascade could be involved in the mediation of this metabolic effect of insulin (Joost and Weber, 1989). In contrast to the effect of insulin or other hormones on amino acid transport insulin's effect on glucose transport seems independent of changes in cAMP concentrations and occurs very rapidly (Kahn et al., 1992). The precise mechanism, however, needs to be elucidated. Recently, Sternlicht et al. showed that exercise and insulin increased glucose transport by different mechanisms (Sternlicht et al., 1989) (Fig. 10). Both GLUT4 and GLUT1 are translocated to plasma membrane upon insulin stimulation, but their individual importance is quantitatively different (Piper et al., 1991).

5º *Activation of Major Target Enzymes*

As mentioned earlier, activation of key enzymes of various metabolic pathways such as carbohydrate, lipid and protein by insulin is brought about mainly through a phosphorylation/dephosphorylation mechanism. Lipid metabolism enzymes ATP citrate lyase and acetyl-CoA carboxylase are phosphorylated by insulin (Denton, 1986). The protein kinase responsible for the insulin-dependent phosphorylation of

acetyl-CoA carboxylase and ATP citrate lyase has yet to be identified. Insulin will activate these two enzymes but the full mechanism remains to be elucidated.

Insulin also controls the activity of some phosphatases responsible for the dephosphorylation of specific proteins involved in some of the hormonal action (Cohen, 1989).

At least five enzymes are known to undergo dephosphorylation in response to insulin stimulation: Glycogen synthase, PDH, PK, hormone sensitive lipase and HMG-CoA reductase. The first three enzymes are involved in glucose metabolism while the last two act on lipid metabolism, lipid mobilization and cholesterol metabolism.

Glycogen synthase exists as an inactive phosphorylated form and as an active dephosphorylated form. Cohen et al. (1985) have contributed enormously to our understanding of glycogen synthase action. Glycogen synthase is activated by a dephosphorylation of one of its sites by a specific protein phosphatase 1 as shown in Figure 11. Glycogen synthase is inactivated by phosphorylation of its three serines moieties by the action of glycogen synthase kinase III and cAMP-dependent-kinase. While phosphorylation of site-5 is without effect by itself, phosphorylation of the latter site is a prerequisite for phosphorylation of sites-3 (Picton et al., 1982; Taylor, 1991). Insulin activation of glycogen synthase *in vivo* results from dephosphorylation of site-3 (Parker et al., 1983).

The PDH complex is a site of multiple regulatory interactions because it lies in a busy "carrefour" which serves as a point of no return for glucose metabolism. Insulin stimulates dephosphorylation of the enzyme complex and this accelerates the conversion of pyruvate into acetyl-CoA (Stryer, 1988). It seems that stimulation of the phosphatase more than the kinase might be implicated in the short term effect of insulin on the PDH complex (Denton, 1986). It has recently been shown by Gottschalk, that the

insulin receptor tyrosine kinase itself does not play an essential role in the insulin signaling pathway that stimulates PDH (Gottschalk, 1991).

PK catalyzes the transformation of PEP into pyruvate. Insulin can override the glucagon inhibition phosphorylation of PK. Although insulin favors the dephosphorylated state of the enzyme, the mechanisms by which insulin acts are not clearly understood (Blair, 1986). It appears that insulin controls L-PK by affecting protein synthesis and increasing the quantity of functional mRNA both of which increase *de novo* synthesis of the enzyme (Evans et al., 1986).

Insulin decreases PEPCK synthesis by inhibiting the transcription of PEPCK gene (O'Brien and Granner, 1990). PEPCK catalyzes the transformation of OAA to PEP, the rate-limiting step in hepatic and renal gluconeogenesis. Moreover, it has been found essential for the synthesis of α-glycerophosphate in adipose tissue. The cytosolic form of the enzyme is influenced by hormonal as well as dietary manipulations. *In vivo*, insulin is the sole hormone which decreased PEPCK activity (Granner and Andreone, 1985).

Insulin inhibits lipolysis by inhibiting hormone sensitive lipase through a dephosphorylation of the enzyme (Stryer, 1988; Unger, 1992). Insulin promotes TG storage by enhancing lipoprotein lipase activity (Bierman, 1972, 1992) as well as protein biosynthesis. Acetyl-CoA carboxylase is stimulated by insulin but the exact mechanism has yet to be resolved. Activation of acetyl-CoA carboxylase and lipoprotein lipase by insulin promote fat synthesis. HMG-CoA reductase, the key enzyme in cholesterol synthesis is also dephosphorylated by insulin. Phosphorylation and dephosphorylation of the reductase through HMG-CoA reductase kinase and HMG-CoA reductase kinase phosphatase are both cAMP-dependent. The exact mechanism is still unclear (Zubay, 1988).

b) Glucagon

1° *Source*

Glucagon is secreted by the α-cell of the islets of Langerhans. Glucagon is a polypeptide of twenty-nine amino acids. Its secretion is mainly controlled by the level of blood glucose as well as by the sympathoadrenal system (Lickley et al., 1983). Catecholamine-induced glucagon secretion is independent of the glucose sensing system (Foster and McGarry, 1988).

2° *Glucagon Receptor*

Glucagon acts through specific receptors whose major functional consequence of ligand occupation is the stimulation, through a stimulatory guanine nucleotide regulatory protein (Gs), of adenyl cyclase activity (Desbuquois and Authier, 1989; Johnson and Dhanasekaran, 1989) (Figs. 12 and 13). Once glucagon is bound to its specific β-adrenergic receptor on hepatic membranes, adenyl cyclase is activated from the effects of the glucagon hormone-receptor complex on a GTP-regulatory protein leading to an increase in cAMP (Kahn et al., 1992). cAMP is a secondary messenger that will bind to the regulatory subunit of the inactive cAMP-dependent protein kinase releasing its catalytic subunit. Catalytic subunit of the activated cAMP-dependent protein kinase is thus ready to phosphorylate many substrates. The activation of the well known cAMP cascade (Sutherland and Park, 1971) is responsible for the major glucagon physiological effects such as stimulation of hepatic glycogenolysis and gluconeogenesis (Chiasson and Cherrington, 1983; Stalmans, 1983). Glucagon could also act through a second type of guanine nucleotide regulatory protein (Gp where p for phospholipase) via a cAMP-independent pathway leading to the breakdown of phosphatidylinositol 4,5-bisphosphate and to the increase in Ca^{++} concentration and activation of a protein kinase C (Exton, 1987; Desbuquois and Authier, 1989).

3° *Target Cells and Enzymes*

At the level of the hepatocytes, glucagon inhibits glycogen synthesis and enhances glucose production through an activation of glycogenolysis and gluconeogenesis (Lefèbvre and Scheen, 1990). All those effects are brought by an increase in intracellular cAMP concentration (Garrison, 1986) which activates a protein kinase A (cAMP-dependent protein kinase), results in phosphorylation of many intracellular proteins of glucose metabolism and enhances hepatic glucose production (Exton, 1987). Protein kinase A phosphorylates the following proteins: 1) phosphorylase *b* kinase which activates GPhase (the rate-limiting step in glycogenolysis); 2) glycogen synthase (the rate-limiting step for glycogenesis) which is inactivated by protein kinase A; 3) L-type PK which is inactivated the enzyme (Blair, 1986), and; 4) the bifunctional enzyme PFK-2/FBPase-2 which results in a decrease in F-2,6-BP, a powerful stimulator of PFK-1 and inhibitor of F-1,6-BPase. The net result is a stimulation of hepatic gluconeogenesis and an inhibition of glycolysis (Fig. 3). The cumulative effect of glucagon on the hepatocytes is a stimulation of hepatic glycogenolysis and gluconeogenesis in parallel with an inhibition of glycogenesis and glycolysis (Hue, 1987; van de Werve and Jeanrenaud, 1987) (Fig. 14).

Glucagon will stimulate gluconeogenesis by augmenting the flux through the rate-limiting reactions of the glucose synthetic pathway. Glucagon stimulation of hepatic gluconeogenesis and inhibition of glycolysis is due to phosphorylation of the bifunctional enzyme PFK-2/FBPase-2. The dephosphorylated form of this enzyme is a kinase while the phosphorylated form is a phosphatase. When glucagon binds to its hepatic receptor, the increase cAMP levels will activate cAMP-dependent protein kinase leading to a phosphorylation of PFK-2/FBPase turning it into a phosphatase and decreasing the level of F-2,6-BP which then inhibits glycolysis and stimulates gluconeogenesis.

The phosphorylation of L-PK by a cAMP-dependent protein kinase by glucagon will modify its kinetic properties resulting in its inhibition (Blair, 1986). However, because allosteric regulation of PK is very strong, high concentrations of F-1,6-BP can overcome the phosphorylation.

Glucagon could also produce longer term effects by affecting the synthesis of enzymes of the gluconeogenesis/glycolysis pathways. An example of glucacon's long term effect is the induction via cAMP-dependent protein kinase of the level of transcription of the PEPCK gene (Claus et al., 1983; Exton, 1987). *In vivo*, glucagon together with glucocorticoids increase the activity of the PEPCK gene (Granner, 1985). It has also been proposed that glucagon could affect the synthesis of L-PK (Evans et al., 1986) by inhibiting its gene transcription.

Glucagon stimulates ketogenesis and blocks hepatic lipogenesis. Both are mediated through a decrease in malonyl-CoA level resulting from an inhibition of glycolysis (as explained in the previous paragraph) and inhibition of acetyl-CoA carboxylase through phosphorylation (Cook et al., 1977). Malonyl-CoA is known to inhibit carnitine palmitoyltransferase I (an enzyme responsible for translocation of impermeable fatty acyl-CoA into mitochondrion); a decrease in the concentration of malonyl-CoA will release the inhibition of the enzyme and accelerate the entry of FA in the mitochondrion. Moreover, it has been shown that glucagon can increase hepatic carnitine levels by an unknown mechanism (McGarry et al., 1975). Carnitine is involved as a fundamental co-factor in the transport of long chain fatty acyl groups into the mitochondria for β-oxidation to acetyl-CoA (Bremer, 1983). An increase in fatty acyl-CoA coupled with an increase in carnitine levels and a stimulation of carnitine palmitoyltransferase I will increase the rate of ketogenesis (McGarry and Foster, 1980). Exercise has also been shown to modify carnitine metabolism (Hiatt et al., 1989).

c) Catecholamines

The role of the catecholamines in the regulation of glucose metabolism has been reviewed by Clutter et al. (1988). The sympathochromaffin system is composed of the adrenal medulla and the sympathetic nervous system (Landsberg and Young, 1992).

1° *Source*

Catecholamines are the hormones secreted by the sympathetic nervous system. In the periphery, norepinephrine is produced at the sympathetic nerve endings. These neurotransmitters can spill over in the general circulation from synaptic clefts adjacent to receptors on target cells after intense activation of the sympathetic nervous system. In the adrenal medulla, epinephrine is the major hormone secreted and it is released in response to stress as it prepares the organism for fight or flight response. The release of catecholamines occurs by exocytosis in a stimulus-secretion coupling. Catecholamines are synthesized from the amino acid tyrosine through the following sequence: tyrosine → dihydroxyphenylaline → dopamine → norepinephrine → epinephrine. The rate-limiting enzyme is the tyrosine hydroxylase. Sympathetic postganglionic nerves and some of the adrenomedullary cells (20%) do not have the enzyme phenylethanolamine N-methyltransferase (PNMT) which converts norepinephrine to epinephrine and therefore the release of norepinephrine is their major secretory product (Landsberg and Young, 1992). Dopamine will be excluded from this short review.

2° *Adrenergic Receptor*

The effects of catecholamines are mediated by α- and β-adrenergic receptors or adrenoreceptors. They are further divided into α_1- and α_2- and β_1- and β_2-

adrenergic receptor subclasses (Clutter et al., 1980; Cryer, 1980; Shah et al., 1984; Goodhardt and Hanoune, 1986; Wang et al., 1991; Landsberg and Young, 1992).

Catecholamine receptors either α or β appear to be intrinsic membrane glycoproteins with a single polypeptide chain (Albert et al., 1989). Their actions are mediated by a guanine nucleotide regulatory protein (G-protein) which is linked to adenylate cyclase. The G-protein involved with both β-adrenergic type receptors is a stimulatory G-protein (Gs) while the α_2-adrenergic receptor act through an inhibitory G-protein (Gi) (Johnson and Dhanasekaran, 1989). The latter leads to a decrease in cAMP concentrations whereas the former increases the concentration of this second messenger. α_1-adrenergic receptors work through another type of G-protein (Gp for phospholipase) which activates a phospholipase C connected to phosphoinositide metabolites.

Norepinephrine as mentioned earlier is primarily a neurotransmitter of sympathetic postganglionic neurons which serves as a hormone when its plasma concentrations reach "biologically effective levels" (Silverberg et al., 1978; Cryer, 1980; Shah et al., 1984).

At the level of the liver, catecholamine stimulation of *Ra* appears to be mediated through α-adrenergic receptors via a cAMP-independent pathway (Goodhart and Hanoune, 1986).

3° *Target Cells and Enzymes*

First, we will consider the effects of catecholamines at the level of the hepatocyte followed by skeletal muscle and then the adipocyte. Depending on the species, catecholamines can induce changes in either cAMP or Ca^{++} levels. The mechanism behind catecholamines regulation of hepatic metabolism include the ones described below.

Catecholamines can stimulate hepatic glucose production through either β_2 and α_1-adrenergic stimulation. Catecholamines through β_2-adrenergic receptors will elicit a response similar to the one described in the section on glucagon via an increase in cAMP levels (Table X). β_2-adrenergic agonists will increase hepatic glucose output via a stimulation of glycogenolysis and gluconeogenesis and via an inhibition of glycolysis and glycogenesis. The stimulation of α_1-adrenergic receptors on the liver result in the increase in IP_3, which acts as an intracellular messenger for Ca^{++} mobilization from the phosphodiesterase-mediated breakdown of the plasma membrane polyphosphoinositide phosphatidylinositol 4,5-P_2 to IP_3 and 1,2-diacylglycerol. The major target for calcium is the calmodulin subunit of some enzymes such as phosphorylase *b* kinase which will activate GPhase and a multisubstrate calcium-calmodulin-dependent protein kinase which phosphorylates and inactivates glycogen synthase and PK (Cherrington et al., 1984; Sherwin and Sacca, 1984; Exton 1987). In humans, hepatic glucose production is mainly the result of a β-adrenergic mechanism (Rizza et al., 1980). Epinephrine will result in hyperglycemia through direct and indirect effects (Fig. 15). The direct action of epinephrine will result in decreased glucose uptake by the liver as well as by the peripheral tissues where skeletal muscle is predominant (Saccà et al., 1982; Bonen et al 1990; Vranic and Lickley, 1990). It will also stimulate hepatic glucose production mainly through β-adrenergic stimulation (Cherrington et al., 1984; Sherwin and Saccà, 1984). Indirect actions represent the α_2-adrenergic inhibition of insulin secretion and the β-adrenergic stimulation of glucagon secretion.

In the muscle, limitation of glucose uptake by catecholamines might come from the initial β-adrenergic stimulation of glycogen breakdown. The increased muscle glycogenolysis leading to a rise in hexose phosphate concentration might inhibit HK and thus, glucose uptake (Chiasson et al., 1981). Based on previous work by Randle (1963, 1964ab), it has also been proposed that lipolysis limits insulin-stimulated glucose

utilization in humans through a citrate-induced inhibition by PFK-1. This leads to an accumulation of G-6-P with subsequent inhibition of HK (Randle, 1963; Randle et al., 1963, 1964ab; Ferrannini et al., 1983). Lastly, ketone bodies have been shown to limit glucose utilization (Wolfe and Shaw, 1984). In the muscle, epinephrine will stimulate glycolysis through a cAMP-dependent protein kinase which phosphorylates a muscle kinase and catalyzes the synthesis of F-2,6-BP the known allosteric activator of PFK-1 (Hue and Rider, 1987; Stryer, 1987; Winder et al., 1991).

Looking at the adipocytes, catecholamines are well known lipolytic hormones. They stimulate lipolysis, increase ketogenesis and decrease net proteolysis resulting in decreased circulating amino acid levels, except for alanine (Clutter et al., 1980).

The lipolytic activity of catecholamines is multifactorial. There is also a direct and indirect lipolytic action of catecholamines (insulin-lowering, glucagon raising). Insulin restricts the lipolytic and ketogenic response to epinephrine. Lipolysis results from the stimulation of hormone sensitive lipase from the increase in cAMP concentration via a β-adrenergic receptor coupling event. Epinephrine is also known for its mobilization of gluconeogenic substrates including lactate, alanine, and glycerol. The consequent increased transport of these substrates from the periphery to the liver is a major factor in the stimulation of hepatic gluconeogenesis by the hormone, although an effect of epinephrine in enhancing the conversion of the various precursors to glucose has also been demonstrated (Cherrington et al., 1984; Stevenson et al 1991).

d) Cortisol

1° *Source*

Glucocorticoids are secreted by the adrenal cortex. Cortisol is the primary glucocorticoid in humans and is essential for life. It is a steroid hormone derived from cholesterol. Cortisol is secreted from the zona fasciculata of the adrenal cortex under the action of ACTH which is in turn controlled by CRH from the hypothalamus (Tepperman, 1980). The hypothalamic-pituitary axis controls adrenal cortical function through the release of ACTH (Kaplan, 1988). After binding to cortex receptors, ACTH activates adenylate cyclase resulting in an increase in cAMP. cAMP activates a cAMP-dependent protein kinase which stimulates the rate-limiting step catalyzed by the 20,22-desmolase reaction leading to an increase in Δ^5-pregnenolone and resulting in the biosynthesis of cortisol (Orth et al., 1992). ACTH is the most important regulator of cortisol secretion. Most of cortisol's effect are permissive with a predominant catabolic action on metabolism. Cortisol is transported in the blood principally bound to corticosteroid-binding globulin (Tepperman, 1980). Less than 10% of cortisol is free, and therefore, active and available for hepatic metabolism and renal excretion.

Cortisol interacts with the brain and the pituitary to stop the release of ACTH and CRH by the hypothalamus. This is bypassed during exercise (emotional and physical stresses) where CRH and ACTH are strongly stimulated despite high blood levels of cortisol (Orth et al., 1992).

2° *Cortisol Receptor*

The glucocorticoid receptor is a phosphoprotein with probably an activity and a function regulated by the state of phosphorylation of the receptor protein (Barnett

and Litwack, 1986). All cortisol actions arise from its binding to specific receptors localized primarily within the nucleus of target cells.

Steroid hormones exert their biological activities by controlling the transcription of tissue-specific genes mediated via high affinity receptor proteins (Barnett and Litwack, 1986).

When the hormone binds to the receptor, it forms an active complex that acquires the capacity to bind specific DNA sequences known as glucocorticoid response elements (GRE) (Beato, 1989) which are adjacent to hormone regulated genes within the nucleus (Schmidt and Litwack, 1982). The binding of the complex steroid hormone-receptor to precise sites modifies the transcription rate, resulting in an augmentation of mature mRNAs of that specific gene through facilitation of the binding of RNA polymerase II to promoter regions of such genes. The mRNAs travel to the cytosol where they bind to ribosomes and are translated into specific proteins. These proteins can in turn modify the metabolic functions of the cell.

The glucocorticoid receptor shares the same general structure as the estrogen and the progesterone receptors. High degree homology can be found in their DNA-binding and their hormone binding regions. The DNA-binding regions have the capacity to insert into a half-turn of DNA and act as transcription factors (Orth et al., 1992). Having to increase or suppress the synthesis of specific enzymes, the delay between cortisol binding and its effect is much longer than in the case of simple allosteric modifications (Clark et al., 1992). The stimulatory response of glucocorticoids are an increase in level and activity of several liver enzymes resulting from an enhanced transcription of mRNAs coding for these enzymes.

The inhibitory responses of glucocorticoids are suppression of DNA synthesis and promotion of skeletal muscle breakdown. These might also involve

glucocorticoid-receptor interactions but the exact mechanism is still unknown (Clark et al., 1992). The binding of the hormone-receptor complex to specific sites on the gene could enhance the synthesis of mRNAs for protein translation that will shut off or inhibit cell function or will simply block DNA transcription (Clark et al., 1992).

3° *Target Cells and Enzymes*

Cortisol affects the liver, muscle and adipose tissue (Fig. 16). Cortisol also affects the lymphoid, skin and connective tissue but these will not be discussed in this text. In addition to a stimulation of hepatic gluconeogenesis, glucocorticoids have a number of inhibitory functions affecting the immune response, glucose uptake by peripheral tissues, secretion of some hormones and neuropeptides, and activity of plasminogen activator and of neutral proteinase (Munck et al., 1984).

Glucocorticoids alter insulin action by modifying receptor and postreceptor function concomitant with a modulation of the tissue response to insulin (McMahon et al., 1988).

Cortisol is also known for its effect on glycogen synthase activity (Hers, 1985). Glucocorticoids augment the activity rather than the amount of the enzyme. Cortisol's effect on liver glycogen metabolism is an activation of glycogen synthase and inactivation of GPhase. It is not known if this action is achieved directly through an activation of a hepatic phosphatase or indirectly through a phosphatase inhibitor (Stalmans and Laloux, 1979).

In the liver, cortisol increases the supply of glucose through gluconeogenesis reflecting the activation of DNA transcription to increase various enzymes involved in the conversion of amino acids to glucose. The following enzymes are affected: G-6-Pase, F-1,6-BPase, PEPCK and PC (McMahon et al., 1988). Cortisol

can also increase hepatic glucose production by enhancing the gluconeogenic and glycogenolytic capacities or by increasing the availability of gluconeogenic precursors. Cortisol increases gluconeogenic substrates availability secondary to an enhancement of the release of the gluconeogenic amino acids from skeletal muscle. Moreover, it activates G-6-Pase and PEPCK through an induced activation of gene transcription mediated by the glucocorticoid-receptor complex with specific GRE for the PEPCK and G-6-Pase genes (Exton, 1979). As mentioned earlier, the presence of glucocorticoids is permissive for the action of other hormones. In the case of lipolysis, glucocorticoids will enhance the sensitivity of lipolysis to catecholamines and other lipolytic hormones, by still unknown molecular mechanisms which bring more glycerol as a gluconeogenic substrate and FFA as an energy source to promote gluconeogenesis (Leboeuf et al., 1962; Fain, 1979).

Glucocorticoids increase amino acid levels in the circulation by stimulating protein breakdown and decreasing the incorporation of amino acids into skeletal muscle proteins (Kaplan and Shimizu, 1963). Cortisol reduces the utilization of amino acids for the formation of proteins everywhere except in the liver where hepatic amino acid uptake is enhanced. The amino acids are channelled for utilization in the gluconeogenic, glycogen formation or protein synthesis pathways.

Glucocorticoids inhibit glucose uptake and utilization from peripheral tissues by direct inhibition brought through a reduction in the number of glucose transporter, mRNA's and transporter number (Fain, 1979). Glucocorticoids impair glucose tolerance by reducing glucose utilization. However, the exact mechanism for the reduction in muscle glucose utilization is still uncertain (Carter-Su and Okamoto, 1985). The reduction of *Rd* could also result from an increase in FFA levels brought about by an enhancement in lipolysis. Citrate accumulation could work as a strong inhibitor of PFK-1 as discussed earlier through the Randle effect (Randle, 1963, 1964; Randle et al., 1964).

Coderre et al. (1992b) have also shown that in the fed state, hypercorticism inhibits skeletal muscle glycogenolysis in response to epinephrine despite GPhase activation and glycogen synthase inactivation.

Finally, glucocorticoids are needed for the complete stimulation of gluconeogenesis by epinephrine and glucagon as demonstrated *in vitro* (Exton et al., 1969) and *in vivo* (Issekutz and Borkow, 1972). The permissive action of cortisol also permits glucagon and epinephrine to act on glycogenolysis and lipolysis (Coderre et al., 1992ab).

e) Growth Hormone

1° *Source*

The effects of growth hormone on carbohydrate and lipid metabolism are direct whereas the growth promoting actions are mediated through a family of peptides (Thorner et al., 1992).

Growth hormone is a polypeptide of 191 amino acids arranged in a single polypeptide chain with more than one disulphide bridges (Luskey, 1988). Growth hormone is released by the anterior pituitary from somatotroph cells in a pulsatile manner by exocytosis into the bloodstream (McCann, 1988). The secretion of growth hormone is under the control of the hypothalamus via the growth hormone releasing hormone (GHRH) and the hypothalamic somatostatin (SRIF somatotropin release-inhibiting factor) which are responsible for the stimulation and the inhibition of growth hormone, respectively (McCann, 1988; Thorner et al., 1992). Thus, the rate at which GH is released by the pituitary will depend on the balance between these two mediators from the hypothalamus. Two other types of stimuli enhance the secretion from the somatotrophs,

one metabolic and the other non-specific such as cold exposure and fight stress including muscular exercise. Growth hormone affects its own secretion via a short loop feedback.

2º *Growth Hormone Receptor*

Human and rabbit growth hormone receptors have been identified as glycoproteins with a unique transmembrane domain that splits the molecule into extracellular and intracytoplasmic domains of equal size (Vinay, 1989; Spencer et al., 1988).

We are still faced by unknown effector systems for the growth hormone receptor, which is known to be unrelated to adenylate cyclase, tyrosine kinase, phosphoinositol turnover and calcium flux as well as ion channels. It might however be related to some cytokine receptors (Kahn et al, 1992; Thorner et al., 1992).

Growth hormone circulates bound to binding proteins. The GH bound to the protein is metabolized differently from monomeric GH and remains in the plasma ten times longer. The biological role of the binding proteins is unclear (Thorner et al., 1992).

It has been shown recently by Bak and colleagues (1991), that growth hormone infusion inhibits glucose oxidation because of an increased lipid oxidation with a concomitant inhibition of the insulin-induced activation of skeletal muscle glycogen synthase. These effects might be compatible with a role for cAMP in the mechanism of GH actions.

3º *Target Cells and Enzymes*

Growth hormone has a number of direct anti-insulin actions resulting in increased lipolysis and increased blood sugar levels (Thorner et al., 1992). Growth hormone can act directly on multiple sites such as muscle, adipose tissue and liver or can

act through somatomedins (IGF-I and IGF-II) for the growth promoting effects of the hormone (Thorner et al., 1992).

Growth hormone is considered as an anabolic, lipolytic and diabetogenic hormone which promotes protein synthesis and lipolysis *in vivo* (Thorner et al., 1992). Continuous GH infusion or pharmacological doses of GH modify carbohydrate (increase blood sugar and anti-insulin effect) and lipid utilization (increase lipolysis) through an induction of insulin resistance at a postreceptor site (Sherwin et al., 1983). In perfused liver, it has been shown that GH causes RNA synthesis, plasma protein synthesis and somatomedin release (Jefferson and Korner, 1967). In isolated rat diaphragm incubation and heart perfusion, GH causes amino acid transport and incorporation (Kostyo et al., 1959; Hjalmarson et al., 1969) while in rat adipocytes, incubation with GH triggers amino acids incorporation and lipolysis (Goodman, 1968).

B. Physiological Regulation of Energy-substrates Metabolism

a) After an Overnight Fast

1° *Hepatic Glucose Production*

As time elapses after a meal, the gradual fall in plasma glucose concentration is associated with a rise in glucagon levels and a decrease in insulin concentrations (Marliss et al., 1970; Exton, 1972; Lopez et al., 1991). The interrelationship between these two pancreatic hormones is crucial to the fine regulation of hepatic glucose production for both glycogenolysis and gluconeogenesis.

We have already discussed the mechanisms of action of glucagon and insulin. In brief, glucagon through its receptor acts via a cAMP cascade to increase GPhase and decrease glycogen synthase activities stimulating glycogenolysis (Stalmans, 1983). The increase in gluconeogenesis is brought about in part by a decrease in F-2,6-BP level as well as a decrease in L-PK and an increase in PEPCK activities. On the other hand, insulin through its receptor increases glycogen synthase activity promoting glycogen synthesis and decreasing glycogenolysis. Inhibition of gluconeogenesis is brought about by an increase in the level of F-2,6-BP, an activation of L-PK and a decrease in PEPCK gene activity. Therefore, glucagon promotes hepatic glucose production from glycogenolysis and gluconeogenesis and insulin reduces hepatic glucose output. A fine balance must prevail between glucagon and insulin in order to maintain an adequate hepatic glucose production.

One of the widely used tool to dissect the role of the pancreatic hormones *in vivo* is SRIF, a polypeptide which inhibits the endogenous secretion of insulin and glucagon (Koerker et al., 1974; D'Alessio et al., 1989). It has been shown by Cherrington and others that the only effect of somatostatin on glucose metabolism is through its dual

inhibition of glucagon and insulin (Altszuler et al., 1976; Cherrington et al., 1977a; Gerich, 1978; Chalmers et al., 1979). Thus, SRIF per se is assumed to have no direct effect on glucose metabolism.

Most of our understanding of the regulation of hepatic glucose metabolism comes from dog studies. In this model, using SRIF, endogenous insulin and glucagon secretion are suppressed. These hormones can then be replaced via the portal vein either singly or in combination and thus, the role of insulin and glucagon on hepatic glucose production can be dissected. The major advantage over human studies is the accessibility of the portal vein (through portal vein catheterization) which is the physiological site of entry for the pancreatic hormones. Thus, this model respects the portal to peripheral ratio in pancreatic hormone concentration.

Working with human subjects, it is important to bear in mind that the portoperipheral ratio of pancreatic hormones is lost (Vranic and Wasserman, 1990). It has been estimated from human studies that the portal to peripheral insulin ratio averages 3.78 (Felig et al., 1974; Berger et al., 1973; Ferrannini and Cobelli, 1987; Shilo et al., 1990) and that the portal to peripheral glucagon gradient is about 1.3-1.7 (Felig et al., 1974). In man, it is clear that with peripheral insulin and glucagon replacement, an improper balance between the liver and periphery exists with respect to these two hormones.

However, it is important to remember that portal glucagon levels are higher than peripheral levels and that portal glucagon levels are pivotal for the action of the hormone on hepatic metabolism. Thus, human studies can still be performed when the aim of glucagon replacement is to reproduce portal levels peripherally. On the other hand, because insulin acts mainly in the periphery, the aim of insulin replacement should be to preferentially duplicate peripheral levels.

De Bodo et al. (1963) were one of the first to show that in the postabsorptive state glucagon was implicated in the process of hepatic glucose production. Chiasson and Cherrington (1983) and Cherrington et al. (1976, 1984, 1977bc) using the SRIF model have shown that the absence of basal glucagon was crucial because it was responsible for almost 75% of the total glucose output in the postabsorptive state. Since glycogen breakdown is the dominant contributor to glucose output, glucagon could be responsible for nearly 90% of the hepatic glycogenolysis after an overnight fast.

While 75% of hepatic glucose production in man is mediated by glucagon (Liljenquist et al., 1977), only about 40% of the total *Rd* occurs in insulin-sensitive tissues. Therefore, if both insulin and glucagon were completely lacking, *Ra* would decrease by 75% to approximately 2.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ or 2.54 g/h, but *Rd* would decrease by only 40% to approximately 5.44 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ or 6 g/h (Unger and Foster, 1992).

From these calculations, we can say from SRIF studies that around 25% of hepatic glucose production is not hormonally-dependent taking together glycogenolysis and gluconeogenesis. When insulin is added jointly to SRIF, results are similar to those obtained with SRIF alone indicating that nearly 75% of basal glucose production is due to glucagon stimulation. When SRIF is infused with glucagon, the absence of insulin results in a 50% increase in hepatic glucose production demonstrating the suppressive effect of basal insulin on hepatic glucose production. These observations demonstrate in dogs that the fine regulation of glycogenolysis and gluconeogenesis depends on the tight interaction of the pancreatic hormones, insulin and glucagon.

Glucagon also affects gluconeogenesis *in vitro* (Exton, 1972, 1979, 1987) and *in vivo* (Chiasson et al., 1974, 1975). *In vivo*, Chiasson et al. (1975, 1975) showed

that the stimulating effect of glucagon on gluconeogenesis can be independent of changes in alanine extraction, indicating that the hormone can stimulate gluconeogenesis intrahepatically. It was also shown that glucagon can stimulate gluconeogenesis by activating key enzymes in the hepatocytes and by stimulating transport of amino acids, mainly alanine, across the plasma membrane (Jennings et al., 1977; Cherrington et al., 1981; Chiasson and Cherrington, 1983, 1984). An increase in glucagon increases hepatic uptake of alanine. Glucagon exerts its effect principally at the liver although a lipolytic effect of glucagon can be demonstrated in adipocytes when supraphysiological concentration of hormone are used (Lefèbvre, 1972). Recently, Jensen et al. (1991) demonstrated that changes in plasma glucagon concentrations within the physiological range had little effect, if any, on adipose tissue lipolysis in humans.

Decreased insulin secretion is critical for the maintenance of postabsorptive plasma glucose concentration because it allows hepatic glucose production to continue on, through hepatic glycogenolysis and gluconeogenesis while glucose uptake is partly inhibited in insulin-sensitive tissues (liver, muscle and fat) sparing glucose for the central nervous system and glycolytic tissues. This is further regulated by the increased glucagon concentration while catecholamines do not seem to play a critical role in the postabsorptive state (Cryer, 1992).

Chiasson et al. (1979, 1980) have shown that higher levels of insulin are needed to suppress gluconeogenesis compared to glycogenolysis. However, a dose-response curve exists between plasma insulin levels and the inhibition of gluconeogenesis (Fig. 17) (Cherrington et al., 1979) since an increase in gluconeogenesis is observed when insulin is made deficient (Jennings et al., 1977; Cherrington et al., 1979). Furthermore, it has been shown by Shilo et al. (1990) that an increasing amount of portal insulin reduced hepatic glucose production (Fig. 18).

Unlike glucagon, insulin works primarily in the periphery by influencing the delivery of gluconeogenic precursors from non-hepatic sources. The decrease in insulin level after an overnight fast favors proteolysis and lipolysis with the release of alanine and glycerol, two gluconeogenic precursors. Insulin thus decreases the quantity of gluconeogenic substrates reaching the liver. Insulin can also affect gluconeogenesis by a direct effect at the liver where basal levels of the hormone exert a restraining effect on the metabolic process. Much higher levels, however, are required to completely inhibit gluconeogenesis (Cherrington and Vranic, 1986).

The extent to which norepinephrine can modify hepatic glucose metabolism still remains poorly characterized. Epinephrine is a well known stimulator of muscle glycogenolysis increasing the activity of the Cori cycle and the glucose-alanine cycle, thereby increasing the supply of lactate and alanine to the liver. Epinephrine and norepinephrine are potent enhancers of lipolysis through an activation of hormone sensitive lipase, resulting in an increased release of glycerol, a gluconeogenic precursor (Landsberg and Young, 1992). In the postabsorptive state *in vivo*, Rizza et al. (1980) and Cherrington et al. (1984) suggested that a physiological rise in epinephrine can augment gluconeogenesis by increasing the delivery of gluconeogenic precursors to the liver. Nevertheless, epinephrine is still ten times less potent than glucagon in stimulating hepatic glucose production (Stevenson et al., 1987). Epinephrine's action on hepatic glucose production differs from that of glucagon. While glucagon elicits its response through a specific receptor linked to adenylate cyclase, epinephrine operates via a specific α_1-adrenergic receptor and calcium mobilization (Exton et al., 1978; Clutter et al., 1988). Using a combination of double radiotracer technique, arteriovenous difference technique and pancreatic clamp, Stevenson et al. (1991) have shown that epinephrine could stimulate progressively both glycogenolysis and gluconeogenesis producing a dose-dependent rise in hepatic glucose production (Fig. 19 and Table X).

Thus, in summary, a decrease in insulin or an increase in epinephrine or glucagon concentrations or both can markedly enhance gluconeogenesis in the postabsorptive state. There is much evidence that an energetic interaction exists between insulin and glucagon in the fine regulation of both glycogenolysis and gluconeogenesis in the intact animal.

2° *Glucose Utilization*

Epinephrine can also inhibit the insulin stimulation of glucose uptake by peripheral tissues (Fig. 15). Raz et al. (1991) recently showed that this effect was mediated, at least in part, by a rise in G-6-P which then inhibits HK. More recently, Christopher et al. (1992) suggested that the effect of epinephrine on glucose uptake was more pronounced in the presence of hyperglycemia and hyperinsulinemia.

In the postabsorptive state, most of the *Rd* (between 60 to 70%) occurs in the non-insulin-dependent tissues such as the brain, the retina, the renal medulla, and the erythrocytes. Glucose uptake in these tissues depends exclusively on glucose concentration via the GLUT1 transporter (Ferré and Girard, 1990). The rest of the *Rd* is by the insulin-dependent tissues, mainly adipocytes and muscle. In these tissues, *Rd* is a function of glucose concentration, insulin concentration as well as insulin sensitivity (Ferré and Girard, 1990). In the basal state, glucose uptake by muscle accounts for only a small portion of the total *Rd* (Jackson et al., 1984) and occurs mainly through GLUT1. Upon stimulation by insulin, glucose uptake occurs mainly through GLUT4 transporters which are translocated from an intracellular pool to the plasma membrane (Klip and Pâquet, 1990). Klip and colleagues (1990) presented data locating GLUT4 in the intracellular membranes and GLUT1 in the plasma membrane.

More than ten years ago, Rizza and colleagues (1979) showed that high doses of epinephrine resulted in impaired glucose clearance in healthy subjects and

suggested that this could be either due to an increase in FFA levels (Randle's effect), to a stimulation of muscle glycogenolysis resulting in an increased G-6-P concentration which inhibits hexokinase or to a direct effect of epinephrine on glucose transporter. It was also shown *in vitro* that epinephrine could directly inhibit muscle glucose uptake (Sloan et al., 1978). More recently, Vranic and Lickley (1990) postulated that insulin and epinephrine work together to control muscle glucose uptake (Fig. 20).

Koranyi and colleagues (1991) demonstrated a strong correlation between insulin-induced whole body glucose uptake during a hyperinsulinemic, euglycemic clamp and the level of muscle GLUT4 transporters indicating an important role for skeletal muscle GLUT4 protein in whole body *Rd*.

b) During Exercise

The factors controlling the stimulation and regulation of hepatic glucose output during exercise remain controversial. Back in 1976, Vranic was already assuming that the regulation of hepatic glucose production during exercise was multifactorial.

1° Hepatic Glucose Production

The role of glucagon in the regulation of hepatic glucose output was first investigated by suppressing the hormone using SRIF. Issekutz and Vranic (1980) showed in dogs that suppression of glucagon by SRIF reduced hepatic glucose production as well as blood glucose and that both could be restored by replacement of the hormone.

Total hepatic glucose production. In man, suppression of glucagon by SRIF during exercise also resulted in hypoglycemia due to a lack of increase in hepatic glucose production (Björkman et al., 1981). Wolfe et al. (1986) examined the role of insulin and glucagon in the regulation of glucose homeostasis during 60 minutes of exercise at nearly 50% VO_2 max. Endogenous glucagon and insulin were suppressed by

the infusion of SRIF, and the hormones were then replaced via a peripheral route to generate normal basal portal vein concentrations during exercise thus preventing the normal exercise-induced modifications in insulin and glucagon levels. They showed that during moderate intensity exercise, the changes in opposite direction of insulin and glucagon are essential to ensure glucose homeostasis during exercise.

Clamping the pancreatic hormones at arterial levels during exercise, Hoelzer et al. (1986) concluded that changes in glucagon and insulin levels were not critical to preserve glucose homeostasis as long as catecholamines were present. Chisholm and Jenkin's groups also studied the regulation of hepatic glucose output in exercising humans (Chisholm et al., 1982; Jenkins et al., 1985, 1986). In the first study they measured glucose turnover during an infusion of insulin at 0.33 mU/kg•min achieving hormonal levels four times basal. They did not find any reduction in hepatic glucose production, probably because the glucagon levels were higher during moderate hyperinsulinemia than during saline infusion (Chisholm et al., 1982). In Jenkin's studies, the authors downplay the role of the pancreatic hormones in regulating *Ra* during exercise and attribute a preponderant role to circulating glucose concentration (1985, 1986). They also suggested that hepatic glucose production was subject to feedback inhibition by circulating glucose without involving the classical glucoregulatory hormones (Jenkins et al., 1986). In another study, Felig et al. (1979) examined the effect of basal insulin and glucagon levels on hepatic *Ra*. They reported that the exercise-induced fall in insulin and/or the exercise-induced rise in glucagon were not fundamental to the increase in glucose production. In that study, however, they did not evaluate separately the impact of either the fall or the rise of insulin or glucagon (Felig and Wahren, 1979). Björkman and coileagues (1988) suggested that during total insulin deficiency, glucose output was under the control of catecholamines rather than glucagon suggesting a role for catecholamine-

induced mobilization of peripheral gluconeogenic precursors and/or hepatic resistance to glucagon.

Vranic and colleagues were one of the first to acknowledge the important role of the increase in glucagon concentration during exercise. Vranic et al. concluded that the role of glucagon was essential in the regulation of glucose output during exercise because its suppression brought inadequate glucose mobilization (1979). When glucagon was suppressed by SRIF, the increase in *Ra* was only half as much that occurring in the normal dog at the end of a 60-minute run (Vranic et al., 1984). Using euglycemic glucose clamp and SRIF infusion, Wasserman et al. (1984) noted that glucagon was responsible for nearly 70% of the increase in *Ra* in exercising dogs. Furthermore, they hypothesized that the glucagon increment during exercise also spared muscle glycogen by enhancing *Ra*. During exercise in the presence of hypoinsulinemia, glucagon could account for 75% of the rise in glucose production while the remaining 25% could be under the control of catecholamines (Wasserman et al., 1984) (Fig. 21).

Ahlborg, Felig and Wahren are in agreement with a role for the fall in insulin and the rise in plasma glucagon during exercise (Wahren et al., 1971; Ahlborg et al., 1974, 1986; Felig et al., 1975; Felig and Wahren, 1975, 1979; Ahlborg and Felig, 1982; Wahren, 1979, 1982). From their studies, they concluded that glucagon contributes to the glycogenolytic and gluconeogenic liver response and that the exercise-induced fall in insulin sensitized hepatic glycogenolysis to glucagon.

More recently, Shilo and his colleagues (1990) studied the effect of 30 minutes of exercise at 40% VO_2 max in healthy and IDDM subjects. In agreement with Wolfe et al. (1986), they showed that in order for *Ra* to increase during exercise, glucagon secretion was essential despite a fixed portal insulin level.

From the discussed studies, it is clear that changes in the levels of glucagon and insulin play a major role in the physiological control of hepatic glucose production during exercise. The opposite effects of exercise-induced changes in the levels of pancreatic hormones control simultaneously both *Ra* pathways. However, as the ratio between glucagon and insulin has a better correlation with hepatic glucose production it might be that the most relevant factor for increasing *Ra* during exercise is the interaction between the changes in glucagon and insulin that is preponderant (Issekutz et al., 1980; Wasserman and Vranic, 1986; Wasserman and Cherrington, 1991).

Both decrements in insulin and increments in glucagon play major roles in the prevention of hypoglycemia during exercise (Hirsh et al., 1989, 1991; Marker et al., 1991). Catecholamines are not normally critical, but progressive hypoglycemia develops when insulin and glucagon are held constant and catecholamine action is blocked (Cryer, 1992).

Gluconeogenesis. Very little information is available on the hormonal regulation of gluconeogenesis during exercise. It has been known for some time, however, that changes occur in gluconeogenic enzyme activities during exercise (Dohm et al., 1985). Others reported that the exercise-induced rise in plasma glucagon and the drop in plasma insulin were both involved in causing the decline in liver F-2,6-BP (Dohm and Newsholme, 1983; Winder, 1985, 1988; Winder et al. 1991). It is well known that insulin counteracts the effects of glucagon on liver F-2,6-BP (Pilkis et al., 1983) although the exact mechanism of insulin's action still remain mysterious.

Based on *in vitro* and *in situ* studies, it is likely that the elevated glucagon/insulin ratio induced by exercise produces a rise in hepatic cAMP which mediates the decline in hepatic F-2,6-BP and orients the metabolic flux in the direction of gluconeogenesis (Winder, 1988). Based on *in vitro* study, Dohm et al. (1983, 1985)

showed that the rise in gluconeogenic flux during exercise resulted from enhanced activities of PC, PEPCK, F-1,6-BPase and G-6-Pase and reduced activities of PFK-1 and PK. All these changes could be mediated initially by the increase in cAMP concentration resulting from the increased concentration of catecholamines, glucagon and from the reduction of insulin observed during exercise (Galbo, 1983; Winder, 1988; Galbo, 1992).

Born and Spratto using adreno-demedullated rats showed that the impairment in gluconeogenesis during exercise was due to the absence of adrenal catecholamines reinforcing the role of epinephrine in the control of gluconeogenesis during exercise (Born and Spratto, 1975).

Very little human studies have been done on the role of hormonal regulation of gluconeogenesis during exercise in healthy subjects. Much of what we know comes from dog studies. Wasserman produced multiple interesting studies where both insulin and glucagon were replaced singly or in combination in the quest for a better understanding of the hormonal regulation of glucose fluxes in exercising dogs (Wasserman et al., 1984, 1985ab, 1988, 1989bcd, 1990, 1991ab). They showed that the glucagon rise during exercise was responsible for nearly 65% of the rise in glucose output resulting from an increase in both glycogenolysis and gluconeogenesis. They also showed that the increase in glucagon was responsible for enhancing gluconeogenic precursors extraction coupled to an increased intrahepatic conversion of alanine to glucose. Wasserman et al. (1989) also showed that the fall in insulin was responsible for nearly 80% of the rise in glucose production during exercise. Their data also suggested that the fall in insulin during exercise could enhance liver sensitivity to glucagon.

2º *Glucose Utilization*

It is quite surprising that during exercise, glucose uptake by muscle varies inversely with the plasma concentration of insulin (Galbo, 1983). *Rd*, therefore, can not

be directly related to insulin concentration. The decrease in plasma insulin combined with an increase in catecholamines prevent an excessive increase in glucose uptake by insulin sensitive tissues during exercise. Catecholamines stimulate muscle glycogen breakdown (Richter, 1984) leading to an accumulation of G-6-P with a concomitant inhibition of HK and intracellular mobilization of FFA (Galbo, 1983). Björkman and colleagues (1988) examined glucose metabolic clearance during exercise (60 minutes at 100 m/min at 10% slope) with total insulin deficiency in insulin-deprived pancreatectomized dogs with or without β-adrenergic blockade. They found that the presence of insulin was not essential for the exercise-induced rise in muscle glucose uptake. They concluded that the main role of insulin during exercise was to limit lipolysis and not to directly control glucose uptake by muscle.

Wasserman and his colleagues (1991c) recently presented a human study in which they showed that insulin and exercise (40 minutes moderate-intensity exercise) worked synergistically to increase glucose uptake, confirming previous results by DeFronzo and colleagues (1981). On the other hand, using depancreatized dogs maintained on constant intraportal basal insulin infusion, Vranic and colleagues (1976) suggested that small amounts of insulin were essential to allow glucose uptake in working muscle.

Although a number of studies have now confirmed that muscle contractions can enhance glucose uptake *in vitro* (Nesher et al., 1985; Ploug et al., 1987, 1992) the precise role of muscle contractions *per se* and insulin *in vivo* still remain to be characterized.

3º *Lipolysis*

Björkman et al. (1988) hypothesized that during exercise insulin restrains lipolysis limiting muscle FFA oxidation. In humans, lipolysis is mainly under the control

of the sympathoadrenal system (Havel and Goldfein, 1959) and insulin (Galbo, 1983; Arner et al., 1990; Warhenberg et al., 1991). As reviewed by Shepherd and Holloszy, lipolysis is under a multifactorial control by which the catecholamines either epinephrine or norepinephrine are well known lipolytic mediators (Shepherd and Bah, 1988; Holloszy, 1990). However, it has been shown that the norepinephrine from the nerve endings within the adipocytes is more important as a stimulus than circulating epinephrine (Gollnick et al., 1970). Adipose tissue lipolysis is also enhanced by ACTH, glucagon (although not within the physiological concentration), growth hormone, glucocorticoids, thyroid hormone and the exercise-induced decrease in insulin level. The exact importance of each of these hormones for the exercise-induced lipid mobilization is still not characterized. It has been shown by Wasserman et al. (1989a), in running dogs, that the exercise-induced fall in insulin is fundamental for the stimulation of lipolysis. With their results and those of Issekutz (1978), it seems that the exercise-induced fall in insulin plus the exercise-induced rise in β-adrenergic agents are needed for the maximum activation of lipolysis to be expressed. Hargreaves and colleagues, showed that muscle glucose uptake is reduced by an increased availability of FFA possibly at the level of glucose transport rather than the Randle's glucose-fatty acid cycle (Hargreaves et al., 1991). More recently, the group of Yki-Jarvinen (1991) provided data confirming the existence of the glucose-FFA cycle in human skeletal muscle where an inhibition of glucose uptake by high FFA levels was inversely proportional to plasma insulin concentrations.

Thus, the major determinants in the stimulation of lipolysis during exercise appear to be the reduced insulin availability and increased sympathoadrenal activity (Galbo, 1983).

RATIONALE

Exercise is an energy demanding process. It has already been shown that during prolonged mild to moderate exercise, blood glucose concentration remains relatively stable because hepatic glucose production increases to meet the increased glucose requirement by the working muscle. Though it has been shown in dogs that the exercise-induced rise in *Ra* is dependent on glucagon, this has been questioned in man. A role for the exercise-induced decreased in insulin levels for the increase in hepatic glucose production has not been shown in man. However, even if the effects of the pancreatic hormones could be overridden by other factors, it does not inexorably mean that they do not play any role in the increases in hepatic glucose production and *Rd* during exercise.

A decrease in plasma insulin and an increase in plasma glucagon are known to occur during intense and/or prolonged exercise. However, it is not established if these modifications in insulin and glucagon secretion are crucial to the accurate moment-to-moment matching of hepatic glucose production to the enhanced glucose uptake by muscle during exercise. Notwithstanding, the precise separate roles of the increment in glucagon and the fall in insulin have seldom been investigated in healthy subjects.

Except for a few dog studies, gluconeogenesis *per se* has never been measured in man during exercise. Some studies have shown an increase in the net hepatic uptake of gluconeogenic precursors. However, the fate of the extracted gluconeogenic precursors is multiple and one can not, therefore, equate hepatic uptake with gluconeogenesis. Furthermore, nothing is known on the hormonal regulation of gluconeogenesis during exercise in healthy postabsorptive subjects. Moreover, the role of the pancreatic hormones are still questioned during exercise in healthy subjects.

With this information, we hypothesize that in healthy subject:

1) The exercise-induced rise in hepatic glucose production is, at least in part dependent on basal pancreatic glucagon and in part on the decrease in circulating insulin.

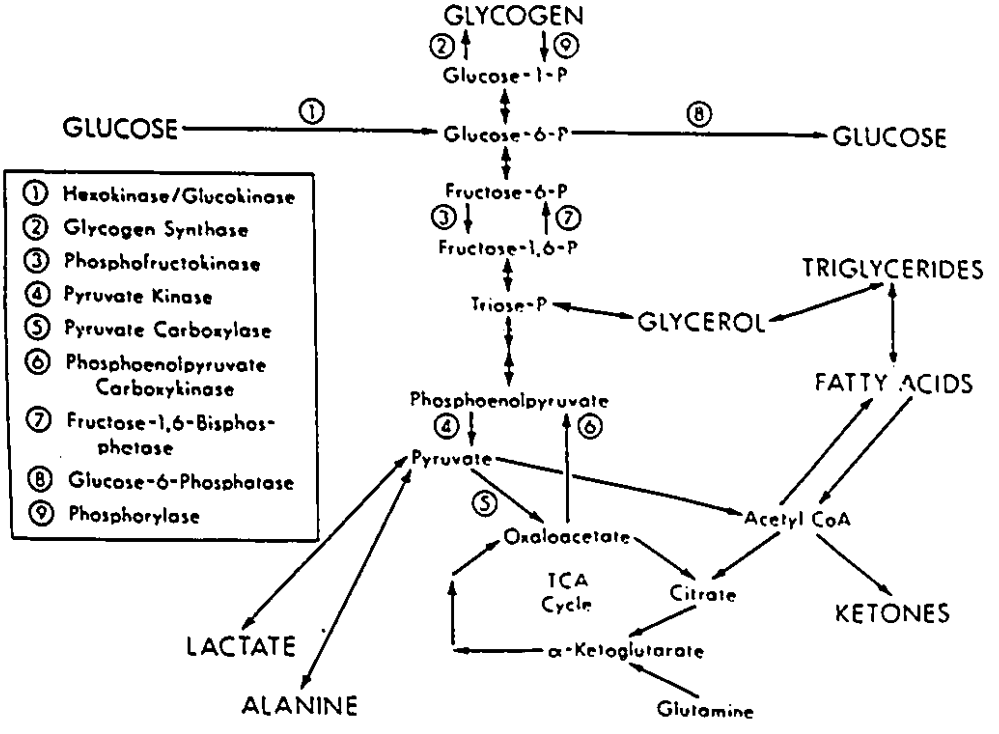
2) The exercise-induced increase in *Rd* is dependent on circulating insulin, the magnitude of which being inversely related to circulating levels of FFA.

OBJECTIVES AND SPECIFIC GOALS

The purpose of this research project was to characterize the regulation of glucose fluxes during mild to moderate exercise in healthy male subjects. Specifically, we wanted to dissect the roles of insulin and glucagon in the regulation of those metabolic processes during mild to moderate exercise using a stable isotope tracer technique to measure: 1) hepatic glucose production; 2) gluconeogenesis; 3) *Rd*; and, 4) lipolysis during a 120-minute ergocycle exercise at 40% VO_2 max.

FIGURE 1

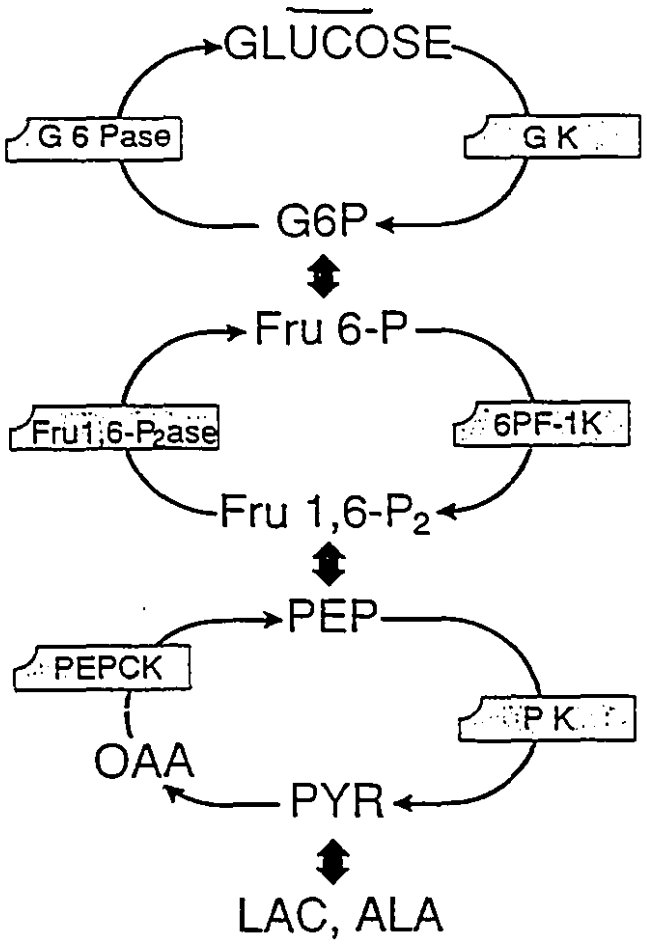
Schematic Representation of an Overview of Carbohydrate Metabolism Showing the Major Interconnections



Adapted from: CRYER, P.E. Glucose homeostasis and hypoglycemia. *In*: Williams Textbook of Endocrinology, Wilson, J.D., and D.W. Foster (Eds.). W.B. Saunders Company, Philadelphia, 1992, 1223-1254.

FIGURE 2

Substrate Cycles in Gluconeogenic Pathway

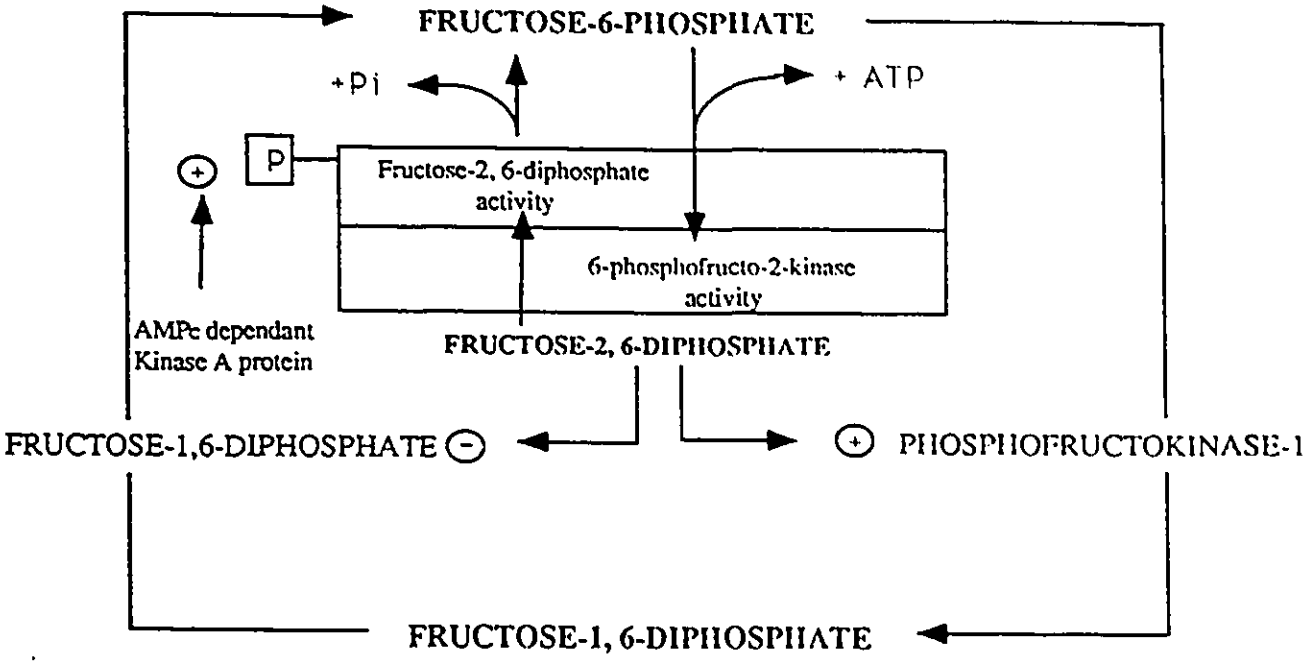


Conversion of PYR (pyruvate) to OAA (oxaloacetate) is catalyzed by mitochondria PC (pyruvate carboxylase). PK: pyruvate kinase; PEPCK: phosphoenolpyruvate carboxykinase; 6PF-1K: 6-phosphofructo-1-kinase; Fru1,6-P_2ase: fructose-1,6-bisphosphatase; G6Pase: glucose-6-phosphatase; GK: glucokinase; ALA: alanine; LAC: lactate; PEP: phosphoenolpyruvate.

Adapted from: PILKIS, S.J., M.R. EL-MAGHRABI, AND T.H. CLAUS. Fructose-2,6-bisphosphate in control of hepatic gluconeogenesis. From metabolites to molecular genetics. *Diabetes Care* 13: 582-599, 1990.

FIGURE 3

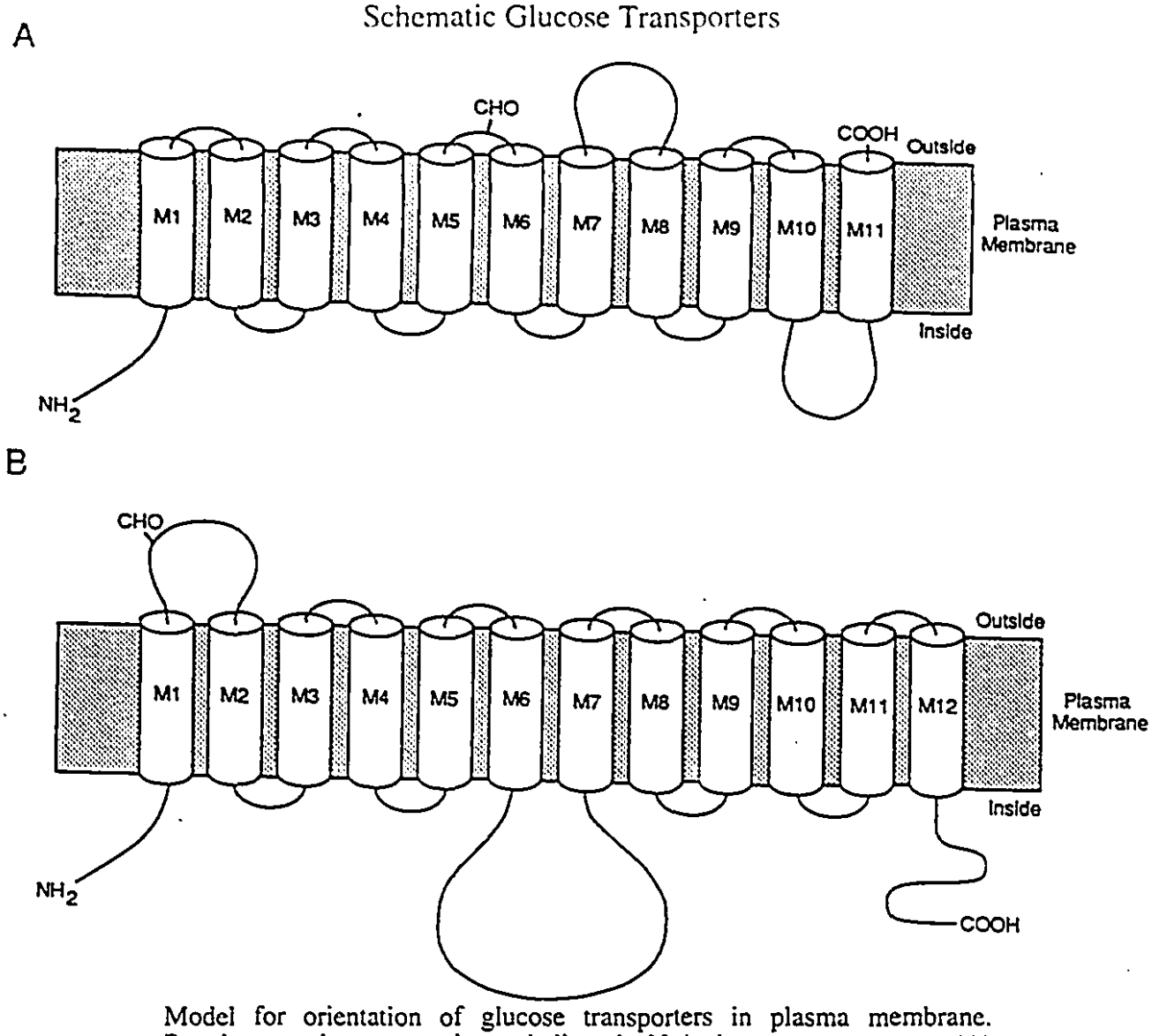
Regulation of Gluconeogenesis at the Level of Fructose-6-Phosphate/Fructose-1,6-bisphosphate



The bifunctional enzyme (PFK-2/FBPase-2) depicted in the box catalyzed depending on its phosphorylation state synthesis or degradation of fructose-2,6-diphosphate.

Adapted from: FERRÉ, P., AND J. GIRARD. Régulation de la glycémie. *Dans:* Traité de Diabétologie, G. Tchobroutsky, G. Slama, R. Assan, and P. Freychet (Éds.) Paris, Editions Pradel, 1990, p. 88-112.

FIGURE 4

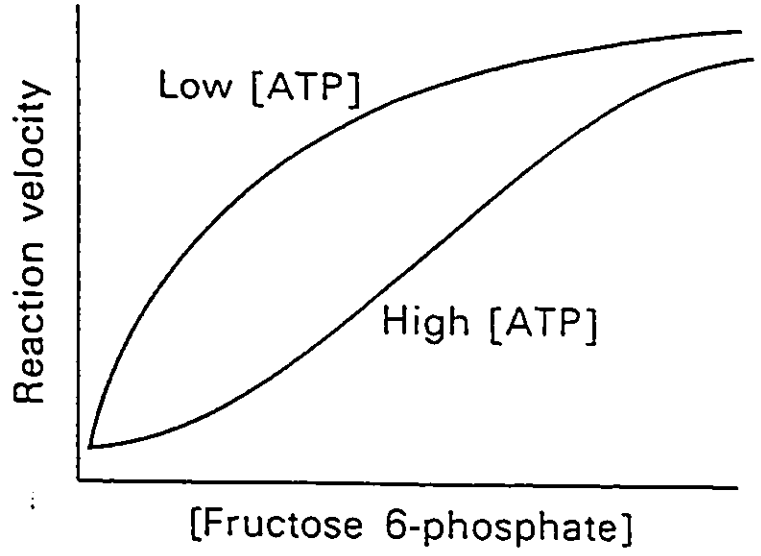


Model for orientation of glucose transporters in plasma membrane. Putative membrane-spanning α-helices in Na^+-glucose-cotransporter (A) and facilitative glucose-transporter; (B) proteins are indicated and numbered M1-M11 and M1-M12, respectively. CHO denotes site of asparagine-linked glycosylation.

Adapted from: BELL, G.I., T. KAYANO, J.B. BUSE, C.F. BURANT, J. TAKEDA, D. LIN, H. FUKUMOTO, AND S. SEINO. Molecular biology of mammalian glucose transporters. *Diabetes Care* 13: 198-208, 1990.

FIGURE 5

Allosteric Regulation of Phosphofructokinase

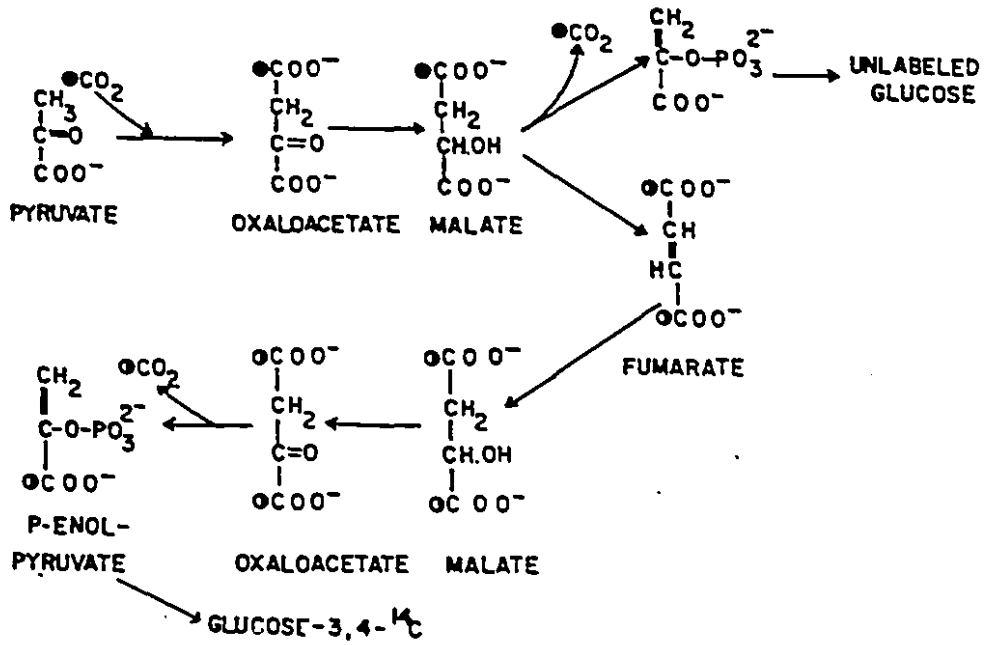


A high level of ATP inhibits the enzyme by decreasing its affinity for fructose 6-phosphate. AMP diminishes and citrate enhances the inhibitory effect of ATP.

Adapted from: STRYER, L. Biochemistry, 3rd Edition. W.H. Freeman and Company, New York, 1988, p. 359.

FIGURE 6

Role of the Equilibration of Labeled Oxaloacetate, Via Malate Dehydrogenase and Fumarase, in the Incorporation of $^{14}CO_2$ into Glucose



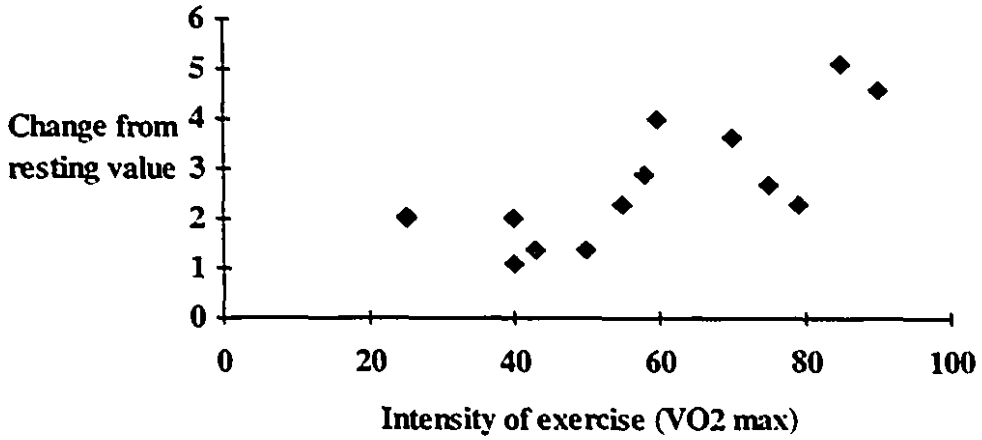
The half-filled symbols indicate the alteration of the specific activity of carbon atoms that occurs as a result of the equilibration.

Adapted from: BROSNAN, J.T. Pathways of carbon flux in gluconeogenesis. *Fed. Proc.* 41: 91-95, 1982.

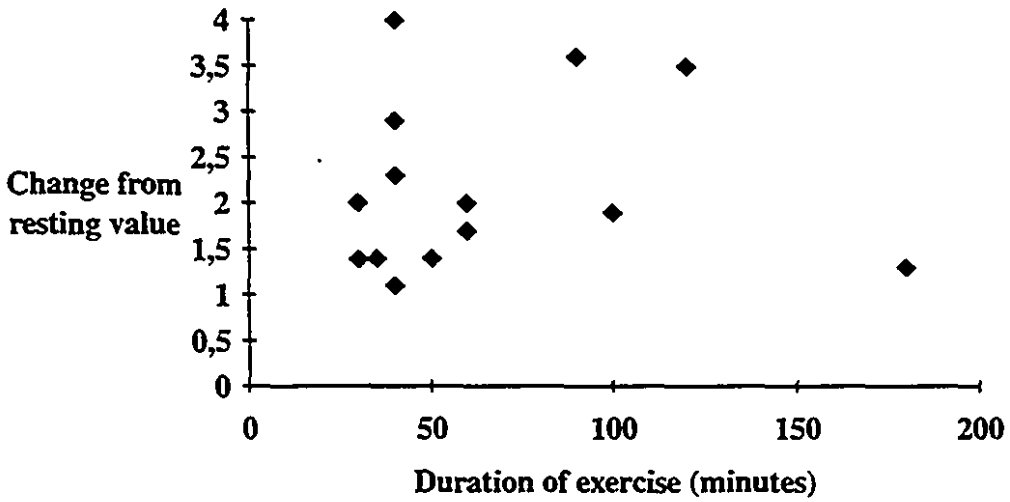
FIGURE 7

Hepatic Glucose Production (*Ra*)

A. Duration of exercise (30 to 60 min)



B. Intensity of exercise (40 to 60% VO2 max)

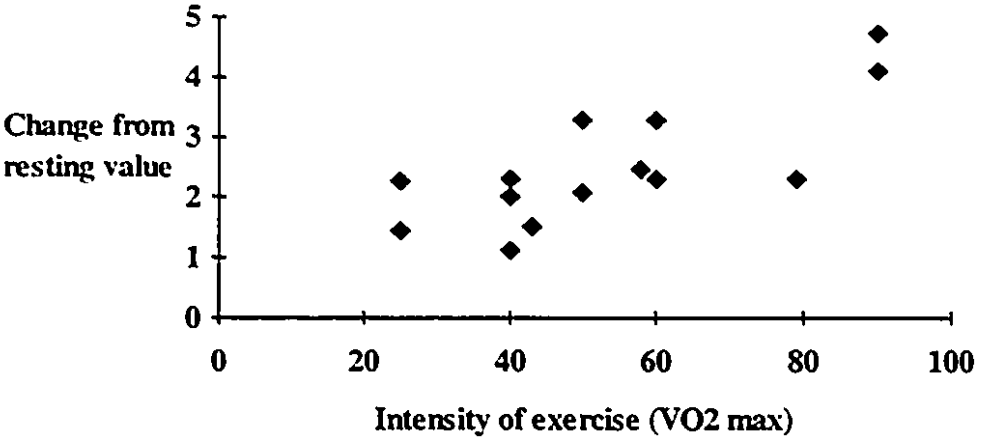


Effect of exercise intensity and duration on hepatic glucose production in postabsorptive state. This graph represents the increase in glucose production (expressed as a delta increase from the resting value) plotted in A against intensity and in B against duration Data were taken from overnight human studies (Wahren et al., 1971; Ahlborg et al., 1974; Wahren et al., 1975; Sestoft et al., 1977; Wahren, 1977; DeFronzo et al., 1981; Stanley et al., 1988; Cooper et al., 1989; Shilo et al., 1990; Weber et al., 1990).

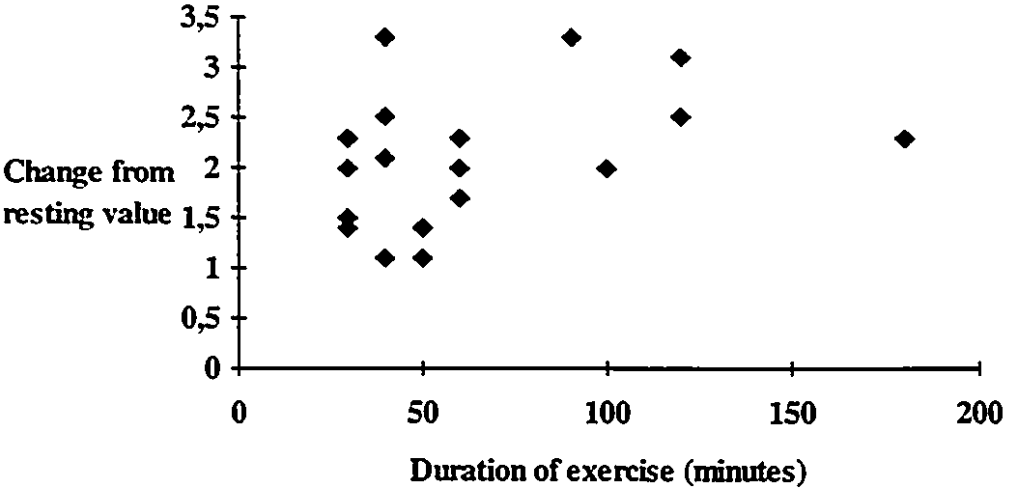
FIGURE 8

Glucose Utilization

A. Duration of exercise (30 to 60 min)



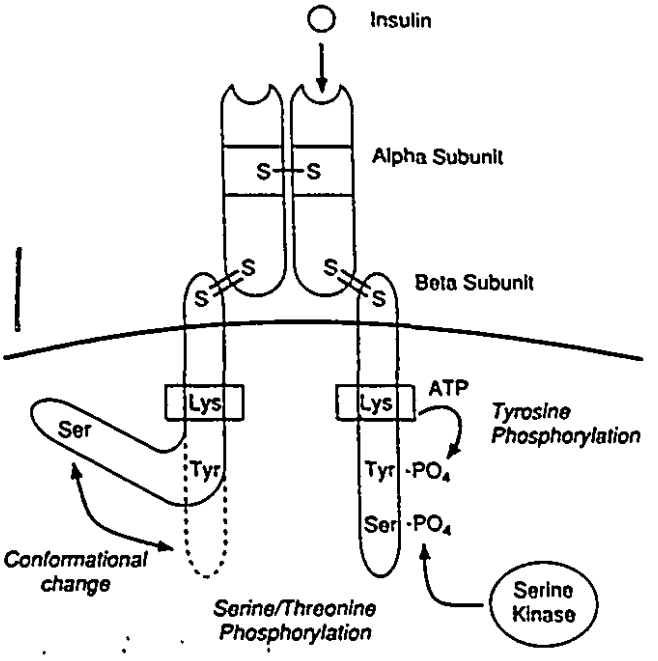
B. Intensity of exercise (40 to 60% VO2 max)



Effect of exercise intensity and duration on glucose utilization in postabsorptive state. This graph represents the increase in glucose uptake (expressed as a delta increase from the resting value) plotted in A against intensity and in B against duration Data were taken from overnight human studies (Wahren et al., 1971; Ahlborg et al., 1974; Wahren et al., 1975; Wahren, 1977; DeFronzo et al., 1981; Jenkins et al., 1985; Stanley et al., 1988; Cooper et al., 1989; Shilo et al., 1990).

FIGURE 9

Insulin Receptor

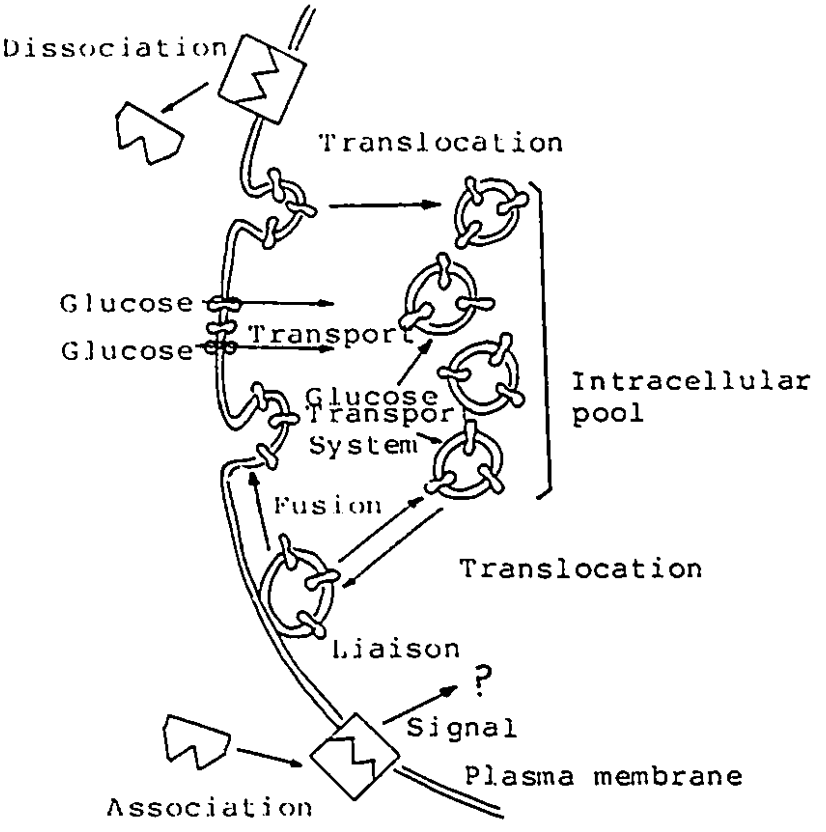


Potential mechanisms for modulation of insulin receptor kinase activity. These mechanisms include tyrosine autophosphorylation, which is stimulatory; serine/threonine phosphorylation by protein kinase C and cAMP kinase, which is inhibitory; and conformational changes, which could produce positive or negative effects.

Adapted from: KAHN, C.R., R.J. SMITH AND W.W. CHIN. Mechanism of action of hormones that act at the cell surface. *In*: Williams Textbook of Endocrinology, J.D. Wilson and D.W. Foster (Eds.). W.B. Saunders Company, Philadelphia,1992, p. 91-134.

FIGURE 10

Insulin Action on Glucose Transporters

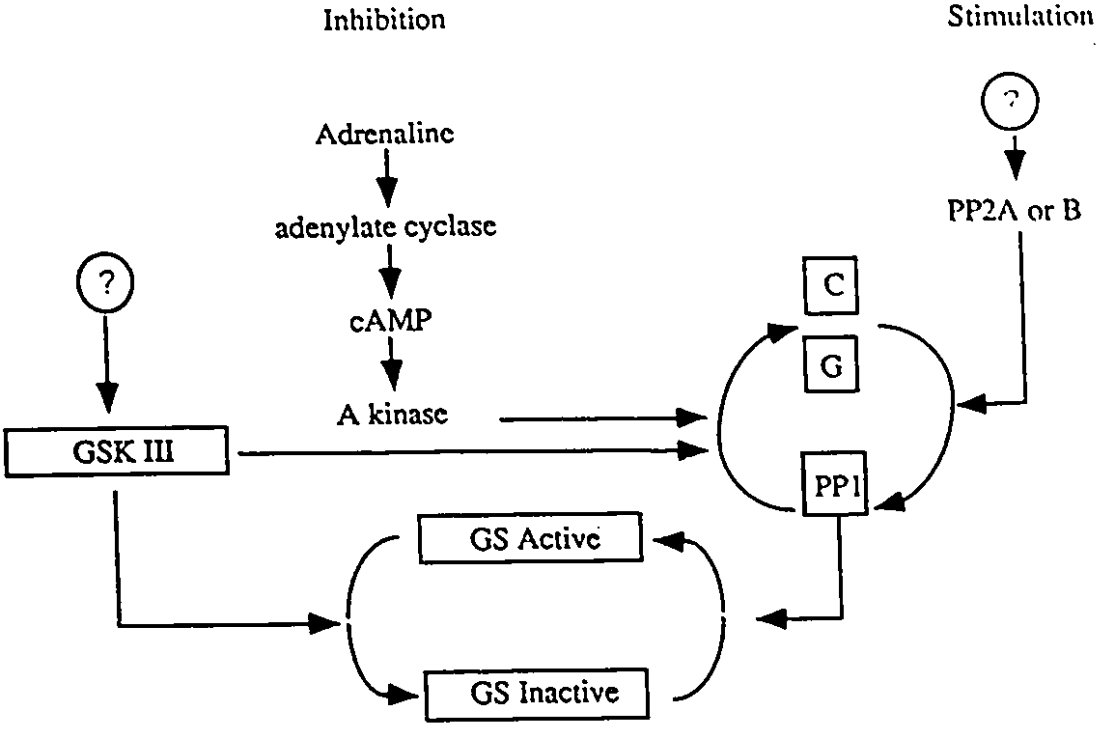


After binding to its receptor, insulin translocate glucose transporters from an intracellular pool to plasma membrane. Upon dissociation between insulin and its receptor, glucose transporters return to their intracellular compartment.

Adapted from: GIRARD, J., AND P. FERRÉ. Mécanismes d'action cellulaire de l'insuline. *Dans*: Traité de Diabétologie, G. Tchobroutsky, G. Slama, R. Assan, and P. Freychet (Éds.) Paris, Éditions Pradel, 1990, p. 141-149.

FIGURE 11

Glycogen Synthase Regulation

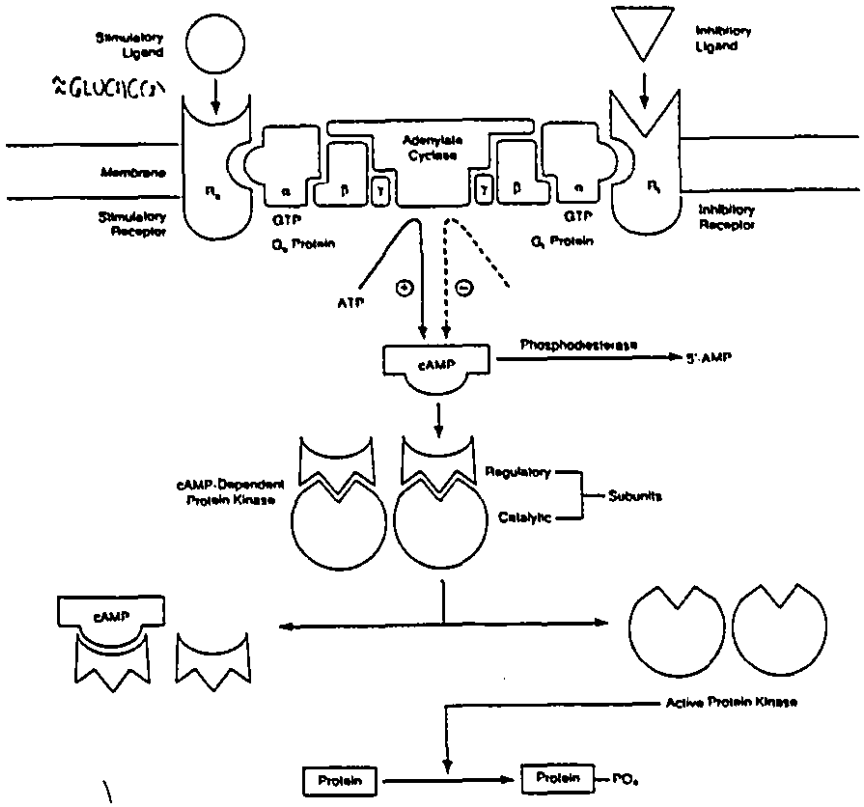


Immediate mechanisms for control of glycogen synthase activity. GS (glycogen synthase) is activated by dephosphorylation of its site 3 serine residues. This is mediated by PP1 (protein phosphatase 1), an enzyme which is activate as a hetero-dimer but inactive when the catalytic (C) and glycogen binding (B) subunits disaggregate. Disaggregation of PP1 is promoted by either GSKIII (glycogen synthase kinase III) or A-kinase phosphorylating the G subunit. Conversely, aggregation and hence activation of PP1 is promoted by PP2A or B (protein phosphatase A2 or B) which causes dephosphorylation of the G subunit. The putative links between insulin receptor activation and PP2 A or B activation are still unknown. GS is inactivated by phosphorylation of its site 3 serines and this is brought about by GSKIII and also by A-kinase. The links between insulin receptor activation and inhibition of GSKIII are still unknown.

Adapted from: TAYLOR, R. Insulin action 1991. *Clin. Endocrinol.* 34: 159-171, 1991.

FIGURE 12

Schematic Depiction of the Adenylate Cyclase System

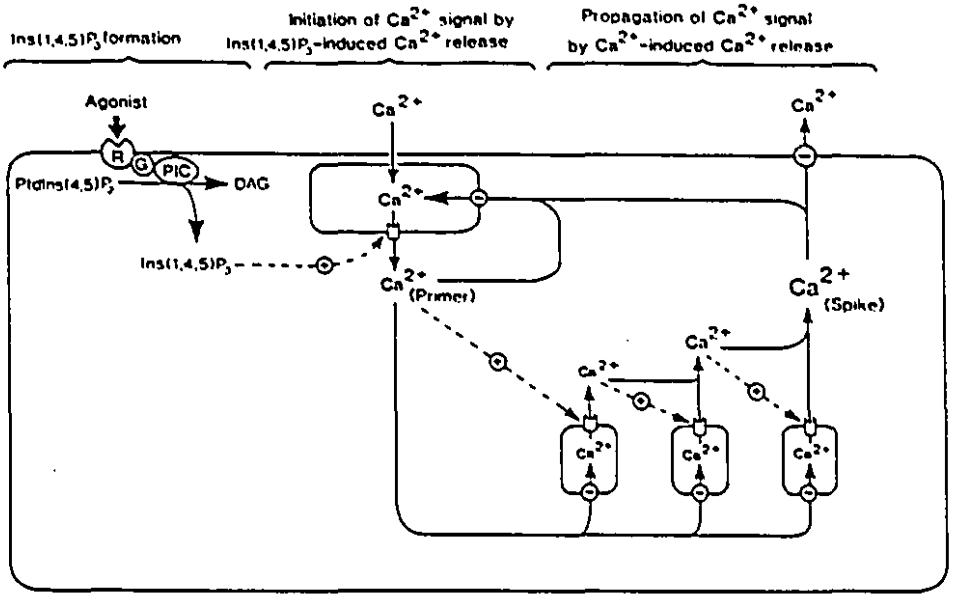


Stimulatory (H_S) and inhibitory (H_i) ligands interact with their respective receptors (R_S and R_i) The complex H_S-R_S activates adenylate cyclase via interaction with the regulatory coupling protein G_S . In the process G_S binds GTP and hydrolyzes it to GDP. The H_i-R_i complex inhibits adenylate cyclase via a homologous regulatory protein G_i. When the catalytic component of adenylate cyclase is activated, ATP is converted to cAMP, which activates cAMP-dependent protein kinase and results in substrate phosphorylation. cAMP-dependent protein kinases consist of four subunits, two catalytic subunits (C) and a dimer regulatory subunit (R-R), which bind two molecules of cAMP. When cAMP binds to the regulatory dimer of the holoenzyme, the catalytic subunits are released and become fully active. With removal of cAMP, the regulatory dimer reassociates with catalytic subunits, inactivating the latter.

Adapted from: KAHN, C.R., R.J. SMITH AND W.W. CHIN. Mechanism of action of hormones that act at the cell surface. *In*: Williams Textbook of Endocrinology, J.D. Wilson and D.W. Foster (Eds.). W.B. Saunders Company, Philadelphia,1992, p. 91-134.

FIGURE 13

IP_3 Stimulation

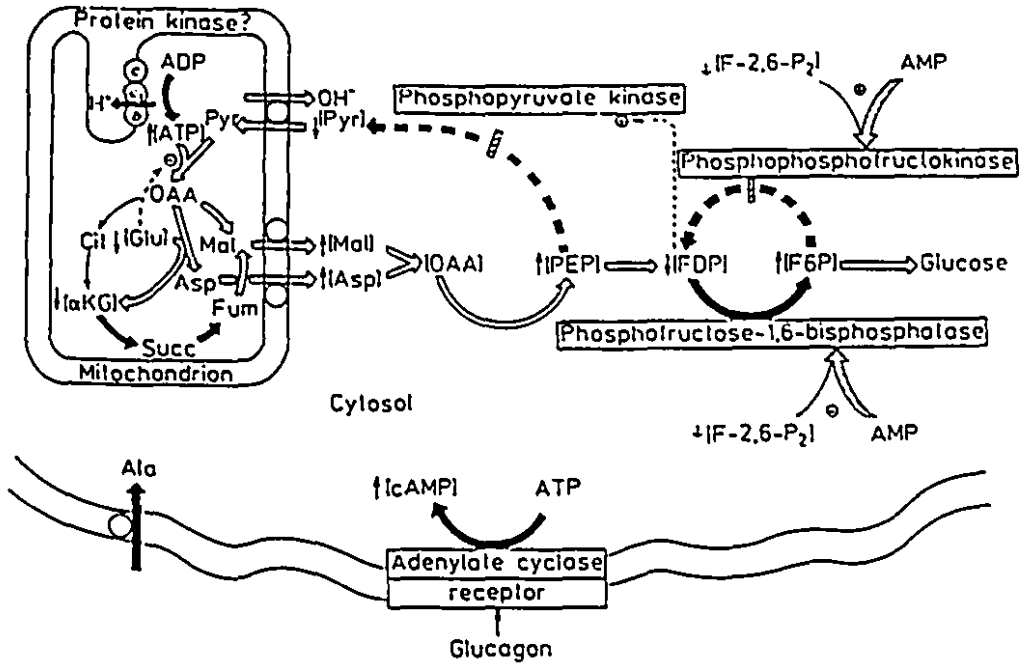


Ca^{2+} (calcium) signaling initiated by the generation of IP_3. Activation of phospholipase C results in an increase in cellular levels of IP_3 ($Ins(1,4,5)P_3$) which then releases calcium from a cytosolic, P_3-sensitive pool and promotes influx of external calcium. Transient elevations in cytosolic calcium may trigger or prime the further release of calcium from P_3-insensitive pools to result in intracellular waves and oscillations.

Adapted from: KAHN, C.R., R.J. SMITH AND W.W. CHIN. Mechanism of action of hormones that act at the cell surface. *In*: Williams Textbook of Endocrinology, J.D. Wilson and D.W. Foster (Eds.). W.B. Saunders Company, Philadelphia,1992, p. 91-134.

FIGURE 14

Changes in the Hepatic Gluconeogenic Pathway in Response to Glucagon

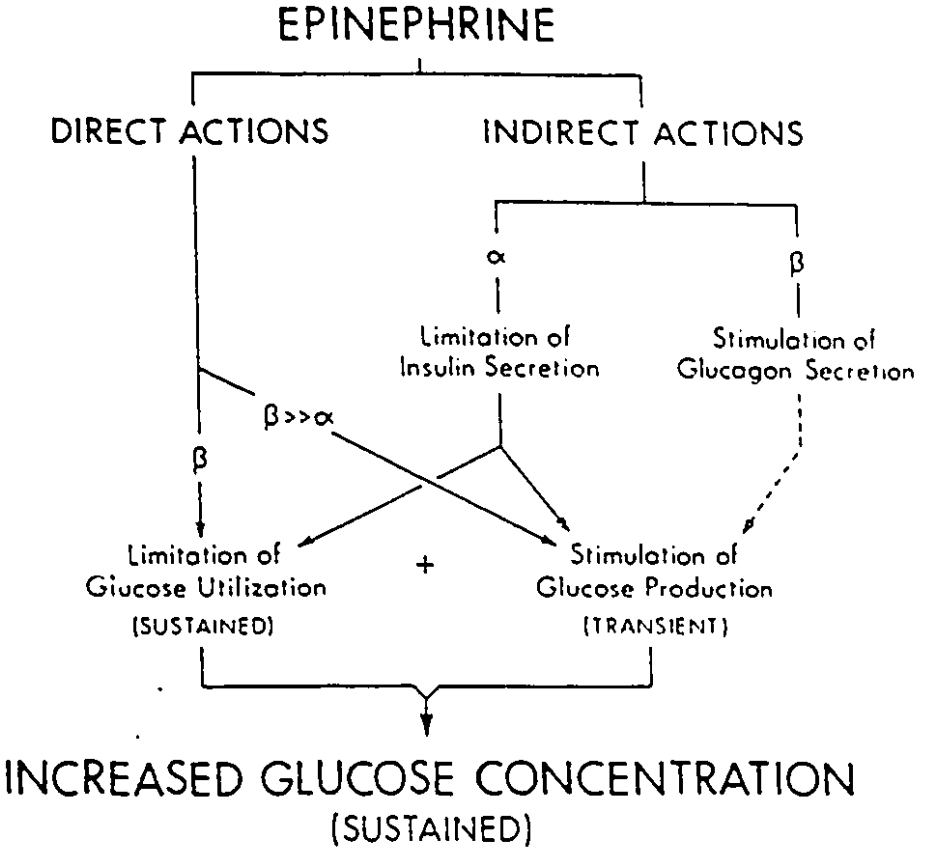


Wavy lines represent the plasma membrane. The rectangle represents the mitochondrion. *Thick open arrows* represent increased flow of substrates. *Thick full arrows* represent sites of glucagon action to accelerate a reaction, while *thick broken arrows* represent sites of action to inhibit a reaction. The *stippled arrows* represent the effect of effectors on enzyme activity. Symbols *b*, *c* and *c1* represent cytochromes. Pyr = pyruvate; Cit = citrate; Mal = malate; αKG = α-ketoglutarate; Succ = succinate; Fum = fumarate; Asp = aspartate; OAA = oxaloacetate; F-2,6-P_2 = fructose-2,6-bisphosphate; PEP = phosphoenolpyruvate; FDP = fructose-1,6-diphosphate; F6P = fructose-6-phosphate.

Adapted from: CLAUS, T.H., C.R. PARK, AND S.J. PILKIS. Glucagon and Gluconeogenesis. *In*: Glucagon I-II, Handbook of Experimental Pharmacology, P.J. Lefèbvre (Ed.). Berlin, Heidelberg, Springer-Verlag, 1983, p. 315-360.

FIGURE 15

Mechanisms of the Hyperglycemic Effect of Epinephrine in Humans

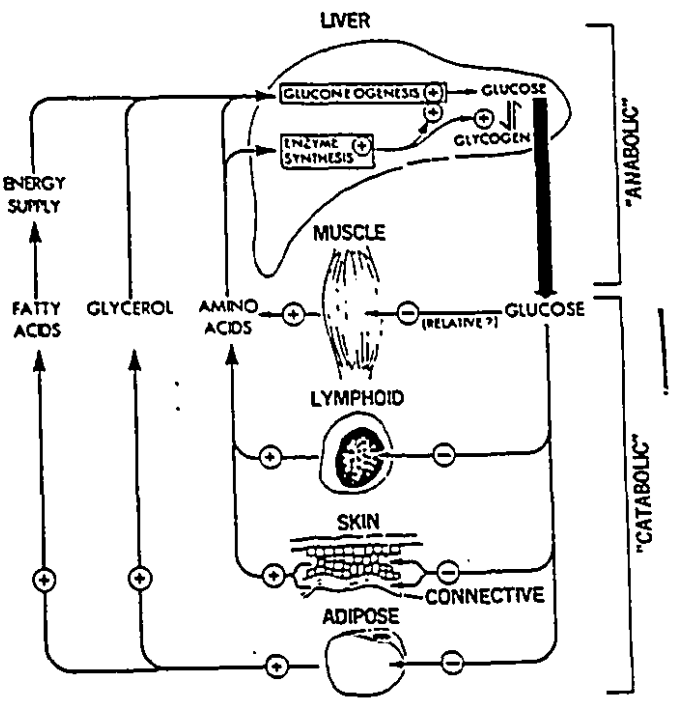


α and β refer to the respective adrenergic receptors.

Adapted from: CLUTTER, W.E., R.A. RIZZA, J.E. GERICH, AND P.E. CRYER. Regulation of glucose metabolism by sympathochromaffin catecholamines. *Diabetes/Metabolism Rev.* 4: 1-15, 1988.

FIGURE 16

Glucocorticoid Effects on Hepatic Glucose Metabolism and Peripheral Tissue Metabolism of Protein and Fat

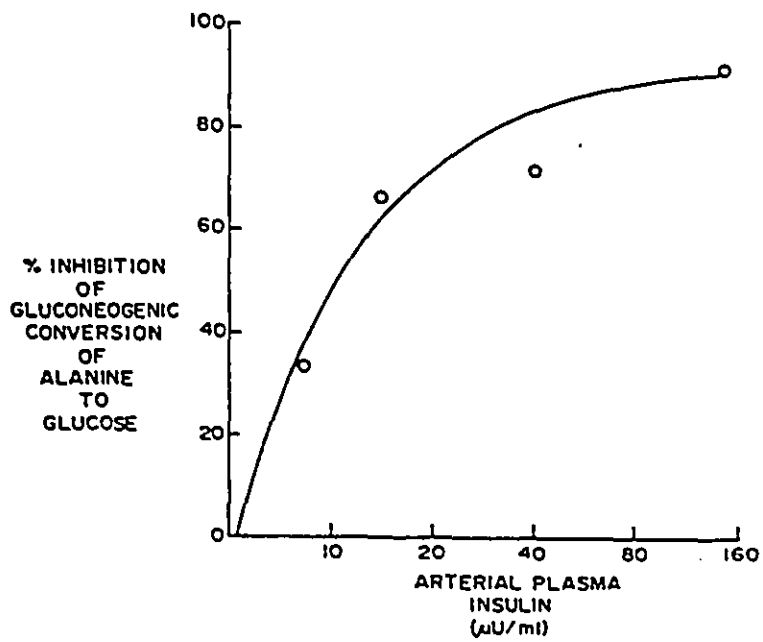


Stimulation is indicated by plus signs and inhibition by minus signs.

Adapted from: ORTH, D.N., W.J. KOVACS, AND C.R. DEBOLD. The adrenal Cortex. *In*: Williams Textbook of Endocrinology, Wilson, J.D., and D.W. Foster (Eds.). W.B. Saunders Company, Philadelphia, 1992, p. 489-620.

FIGURE 17

Schematic Representation of the Ability of Insulin to Inhibit Gluconeogenesis in Overnight Fasted Conscious Dogs

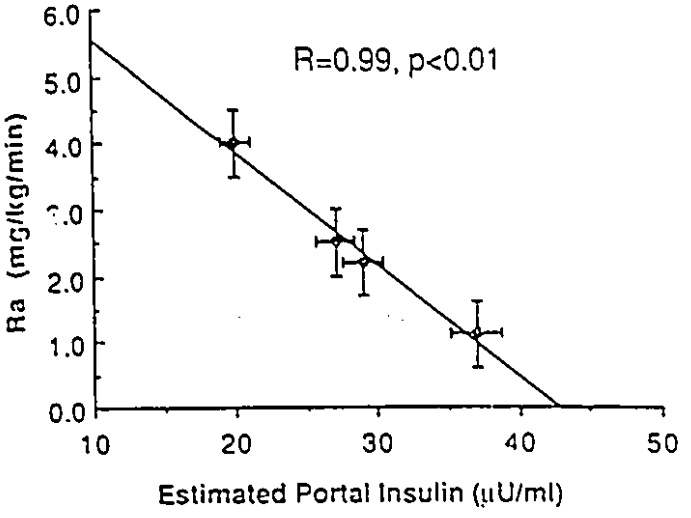


The insulin levels shown are those measured in arterial blood even though insulin was infused intraportally. Glucagon level was fixed at a basal value (50 to 100 pg/ml) in each study using somatostatin and replacement glucagon infusions.

Adapted from: CHERRINGTON, A.D., AND M. VRANIC. Hormonal regulation of gluconeogenesis *in vivo*. *In*: Hormonal Control of Gluconeogenesis: Function and Experimental Approaches, N. Kraus-Friedmann. Boca Raton, Florida, CRC Press, Inc., p. 15-37.

FIGURE 18

Hepatic Glucose Production and Portal Insulin Concentration

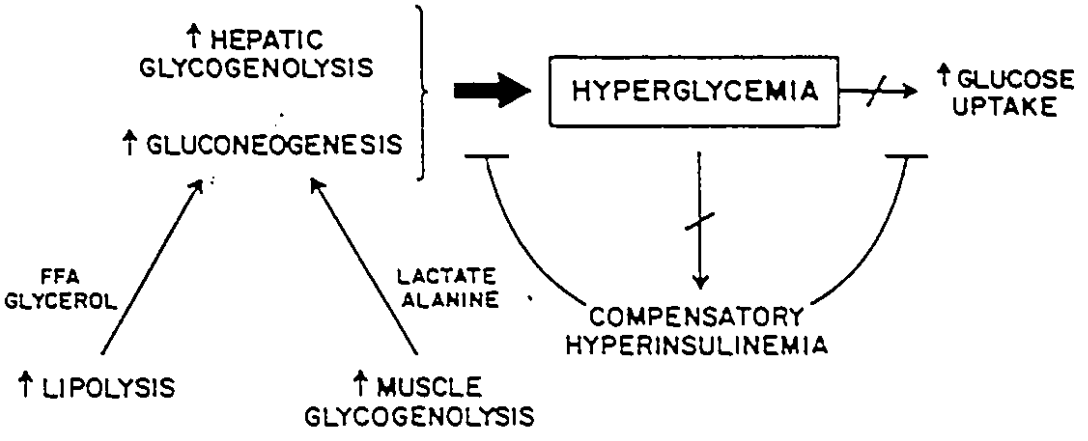


Under conditions of SRIF infusion with fixed glucagon concentration, the response of the liver to exercise is determined by ambient portal insulinemia. Mean data from each of the two insulin infusion doses in normals and IDDM are plotted.

Adapted from: SHILO, S., M. SOTSKY, AND H. SHAMOON. Islet hormonal regulation of glucose turnover during exercise in type 1 diabetes. *J. Clin. Endocrinol. Metab.* 70: 162-171, 1990.

FIGURE 19

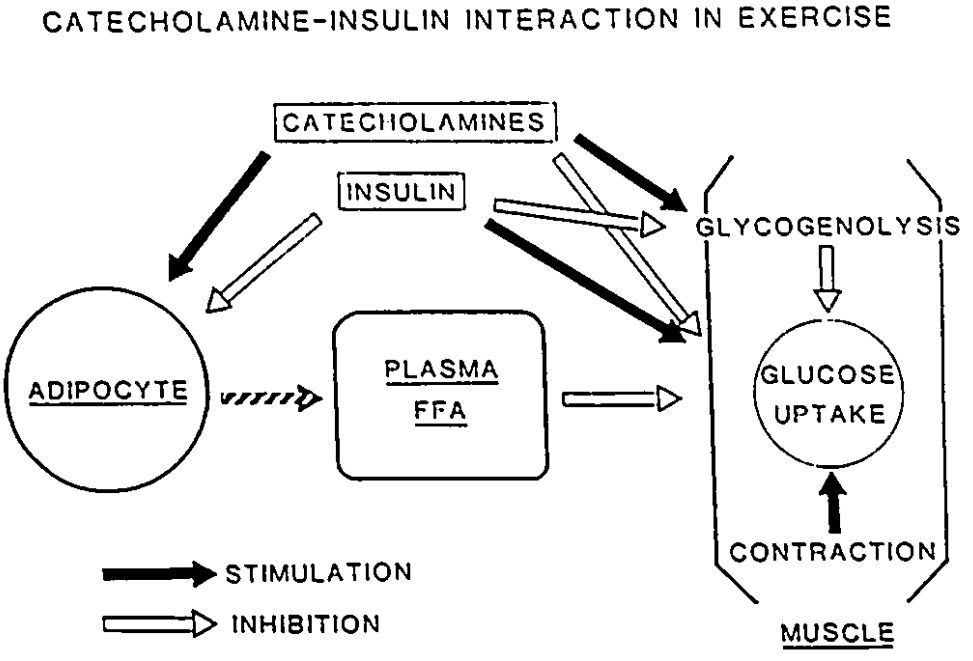
Mechanisms of Hyperglycemic Effect of Epinephrine in Postabsorptive Humans



Adapted from: SHERWIN, R.S. AND L. SACCA. Effect of epinephrine on glucose metabolism in humans: contribution of the liver. *Am. J. Physiol.* 247: E157-E165, 1984.

FIGURE 20

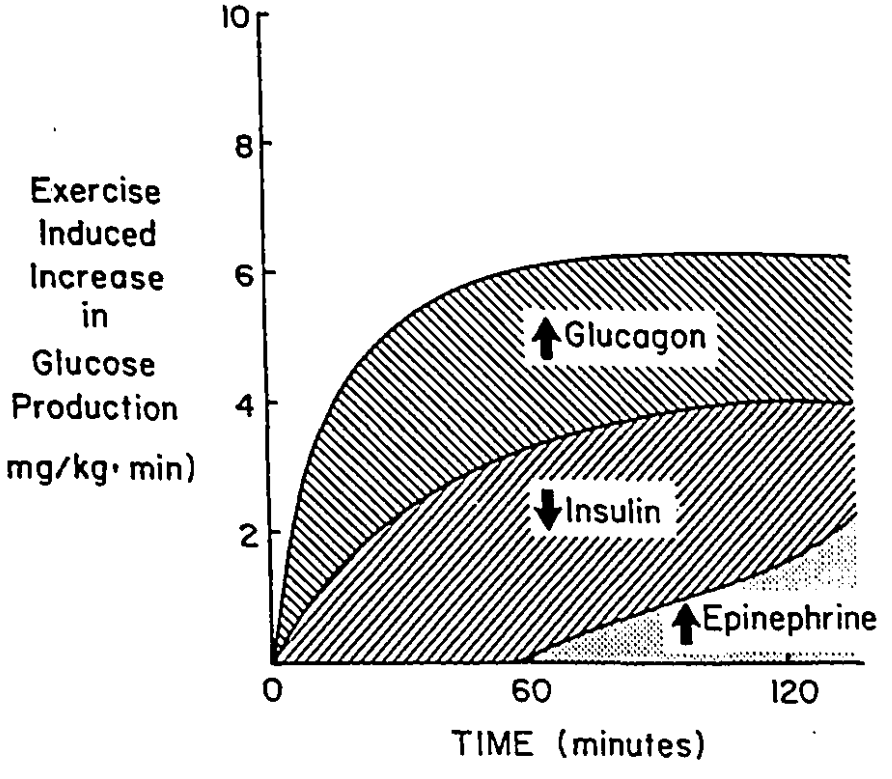
Interaction Between Catecholamines and Insulin in Control of Glucose Uptake



Adapted from: VRANIC, M., AND H.L.A. LICKLEY. Hormonal mechanisms that act to preserve glucose homeostasis during exercise: Two controversial issues. *In*: Biochemistry of Exercise VII. International Series on Sport Sciences, A.W. Taylor, P.D. Gollnick, H.J. Green, C.D. Ianuzzo, E.G. Noble, G. Métivier, and J.R. Sutton (Eds.). Champaign, IL, Human Kinetics Publishers, Inc., 1990a, p. 279-294.

FIGURE 21

Hormonal Regulation of Hepatic Glucose Output During Exercise



Schematic representation of rise in glucose production during moderate-intensity exercise and impact of fall in insulin and rise in glucagon and epinephrine on this response.

Adapted from: WASSERMAN, D.H., AND A.D. CHERRINGTON. Hepatic fuel metabolism during muscular work:role and regulation. *Am. J. Physiol.* 260: E811-E824, 1991a.

TABLE I

Summary of Hormonal Regulation of Gluconeogenesis L-type PK, 6PF-2-K/Fru-2,6-P2ase, and Fru-2,6-P2 levels

Hormone	Gluconeogenesis	Fru-2,6-P_2	Pyruvate kinase	6PF-2-K/FRU-2,6-P_2ase	Mechanism
Glucagon	Stimulates	↓	Inhibits	Inhibits activates	↑ cAMP / ↑ cAMP-protein kinase
Insulin	Inhibits	↑	Activates	Activates/inhibits	↓ cAMP / ↓ cAMP-protein kinase and ↑ protein p'ase
Catecholamines					
β-agonist	Stimulates	↓	Inhibits	Inhibits activates	↑ cAMP / ↑ cAMP-protein kinase
α-agonist	Stimulates	↑	Inhibits	— —	↑ Ca^{2+} / ↑ Ca^{2+} CAM-protein kinase
Vasopressin	Stimulates	↑	Inhibits	— —	↑ Ca^{2+} / ↑ Ca^{2+}/CAM-protein kinase
Angiotensin	Stimulates	↑	Inhibits	— —	↑ Ca^{2+} / ↑ Ca^{2+}/CAM-protein kinase

*(—) indicates no effect on the phosphorylation state of 6PF-2-K/Fru-2,6-P_2ase. Fru-2,6-P_2 levels increase upon addition of α-agonists, vasopressin, and angiotensin because glycogen breakdown is enhanced by a Ca^{2+}-linked mechanism leading to elevated glycolysis and ipso facto elevated Fru-6-P.

Adapted from: PILKIS, S.J., AND T.H. CLAUS. Hepatic gluconeogenesis/glycolysis: regulation and structure/function relationships of substrate cycle enzymes. *Annu. Rev. Nutr.* 11: 465-515, 1991.

TABLE II

Human Glucose Transporters

Designation	Size (n amino acids)	Major sites of expression	Chromosomal location of gene
SGLT1 (Na^+-glucose cotransporter)	664	Small intestine	22q11.2→qter
Facilitative glucose transporters			
GLUT1 (erythrocyte)	492	Placenta, brain, kidney, and colon	1p35→31.3
GLUT2 (liver)	524	Liver, β-cell, kidney, and small intestine	3q26
GLUT3 (brain)	496	Many tissues, including brain, placenta, and kidney	12p13
GLUT4 (muscle/fat)	509	Skeletal muscle, heart, and brown and white fat	17p13
GLUT5 (small intestine)	501	Small intestine (jejunum)	1p31

Adapted from: BELL, G.I., T. KAYANO, J.B. BUSE, C.F. BURANT, J. TAKEDA, D. LIN, H. FUKUMOTO, AND S. SEINO. Molecular biology of mammalian glucose transporters. *Diabetes Care* 13: 198-208, 1990.

TABLE III

Postabsorptive Hepatic Glucose Production (*Ra*)*

TECHNIQUES	AUTHORS	RESULTS
Arteriovenous catheter		
Hepatic vein catheter	Ahlborg et al., 1974	10.79
	Björkman et al., 1981	9.96
	Cahill and Owen, 1968	10.81 to 18.92
	Wahren, 1975, 1978	8.16 to 13.37
	Wahren et al., 1979	8.16 to 13.37
Isotopic dilution		
Radioactive tracers		
[6-^{14}C]-glucose	Virkamäki et al., 1990	10.3 ± 0.5
[3-^{3}H]-glucose	Clore et al., 1991a	11.6 ± 1.1
	Jackson et al., 1984	13.11 ± 0.77
	Jenkins et al., 1985	13.20 ± 0.61
	Levy et al., 1989	11.4 ± 0.7
	Rizza et al., 1979, 1980	10.55 ± 0.55
	Saccà et al., 1979b	10.89 ± 0.72 to 11.22 ± 0.55
[6-^{3}H]-glucose	Consoli and Nurjhan, 1990b	
	Consoli et al., 1987	12.52 ± 0.50
	Consoli et al., 1990a	11.69 ± 0.14
	Lecavalier et al., 1989	12.8 ± 0.4
	Lecavalier et al., 1990	11.0 ± 0.7
	Virkamäki et al., 1990	11.7 ± 0.4
Stable Isotopes		
[6,6-^{2}H]-glucose	Cobelli and Ferrannini, 1987	13.39 ± 0.33
	Knapick et al., 1989	8.9
	McMahon et al., 1988	11.11 ± 1.11
	Reinauer et al., 1990	13.44 ± 0.61
[2,3,4,6,6-$^{2}H_5$]-glucose	Lecavalier, 1987	7.76 ± 0.39
	Martineau et al., 1985	7.3
[U-^{13}C]-glucose	Tserng and Kalhan, 1983	11.22 ± 1.05
Arteriovenous difference and radioactive tracer		
[3-^{3}H]-glucose	DeFronzo and Ferrannini, 1987	12.22 to 12.78

*This table contains only data from 12-14 hour overnight fasted human studies. Results are expressed in $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

TABLE IV

Postabsorptive Gluconeogenesis (*Ra*)*

TECHNIQUES	AUTHORS	RESULTS
Arteriovenous catheter		
Hepatic vein catheter	Ahlborg et al., 1974 Wahren et al., 1975, 1978	2.70 2.55
Isotopic dilution		
[3-^{14}C]-lactate	Consoli and Nurjhan, 1990b	3.57 ± 0.38
[2-^{14}C]-acetate		
[3-^{13}C]-alanine		
[6-^{3}H]-glucose		
[2,3,4,6,6-$^{2}H_5$]-glucose	Lecavalier, 1987	0.510 ± 0.035
[1,2,3-$^{13}C_5$]-alanine		
[2,3,4,6,6-$^{2}H_5$]-glucose	Martineau et al., 1985	0.42
[1,2,3-$^{13}C_5$]-alanine		
[6-^{3}H]-glucose	Lecavalier et al., 1990	1.8 ± 0.4
[U-^{14}C]-lactate		

*This table contains only data from 12-14 hour overnight fasted human studies. Results are expressed in $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

TABLE V

Dog Postabsorptive Hepatic Glucose Production (*Ra*)*

TECHNIQUES	AUTHORS	RESULTS
Radioactive tracers		
[3-^{3}H]-glucose	Myers et al., 1991	15.39 ± 1.05
	Saccà et al., 1978; 1979a	11.22 ± 1.39
+ arteriovenous	Björkman et al., 1988	37.78 ± 6.11
	Issekutz et al., 1967	18.39 ± 1.22
	Issekutz and Vranic, 1980	18.39 ± 1.22
	Issekutz, 1981	18.39 ± 1.22
	Stevenson et al., 1991	13.44 ± 2.67
	Wasserman et al., 1984	24.83 ± 1.72
	Wasserman et al., 1985a	23.33 ± 2.78
	Wasserman et al., 1988, 1989ac	18.33 ± 1.11
	Wasserman et al., 1990	17.78 ± 1.11
[6-^{3}H]-glucose	Radziuk et al., 1978	13.55
[1-^{14}C]-glucose	Radziuk et al., 1978	20.55
[U-^{14}C]-glucose	Vranic et al., 1976a	13.72 ± 1.0

*This table contains only data from 12-14 hour overnight fasted dog studies. Results are expressed in $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

TABLE VI

Exercise and Hepatic Glucose Production (*Ra*)*

INTENSITY %VO_2 MAX				
Duration (minutes)	**20-40**	**41-60**	**61-80**	**≥81**
30-60	2.0x: DeFronzo & al.1981	1.1x: Cooper & al, 1989	2.3x:Cooper & al, 1989	4.6x: Wahren & al, 1971
	2.0x: Wahren, 1977	1.4x: Sestoft & al, 1977	2.7x: Wahren & al, 1971	5.1x: Wahren, 1977
	2.1x: Wahren & al, 1971	1.4x: Stanley & al, 1988		
		1.7x: Jenkins & al, 1986		
		2.0x: Jenkins & al, 1986		
		2.0x: Shilo & al, 1990		
		2.3x: Wahren, 1977		
		2.9x: Ahlborg & Felig, 1982		
		3.0x: Björkman & al, 1988		
		4.0x: Wahren & al, 1975		
61-90		3.6x: Ahlborg & Felig, 1983	3.65x: Weber et al., 1990	
91-120		1.9x: Wasserman & al, 1991		
		3.5x: Ahlborg & Felig, 1983		
121-150		2.5x: Knapik & al, 1988		
≥151	1.8x: Ahlborg & al, 1974	1.3x: Ahlborg & Felig, 1983		

*This table contains only data from 12-14 overnight fasted human studies. These data represent the increase in hepatic glucose production from the resting value reported in the same paper.

TABLE VII

Exercise and Gluconeogenesis⌖

AUTHORS	EXERCISE		RESULTS
	Intensity (%$vo_{2\,max}$)	Duration (min)	gng as % total *Ra*
Allborg et al., 1974*	30	240	45 (+20%)**
Sestoft et al., 1977 Σ gng precursors	50	35	59 (rest to exercise)
Stanley et al., 1988 from ^{14}C-lactate	43 ± 5	30 50	25 (+12.5%) 22 (+9.5%)
Wahren et al., 1971*	25-30	40	16.5 (-8.8%)
Wahren et al., 1971*	50-60	40	10.6 (-14.17%)
Wahren et al., 1971	75-90	40	5.9 (-19.4%)
Wahren et al., 1975	55-60	40	9 (-22%)
Wahren, 1977*	30	40 240	23 (-2%) 45 (+20%)

⌖This table contains only data from 12-14 hour overnight fasted human studies. Results are expressed in $\mu mol \bullet kg^{-1} \bullet min^{-1}$.

*These studies presented gluconeogenesis as estimation from substrate balance across splanchnic bed using the arteriovenous difference catheter technique.

**Parentheses indicate the difference in gluconeogenesis from resting value within the same paper.

TABLE VIII

Exercise and Glucose Utilization (*Rd*)*

Intensity % VO_2 max				
Duration (minutes)	**20-40**	**41-60**	**61-80**	**≥81**
30-60	7.0x: Wahren, 1977†	1.1x: Cooper & al. 1989‡	2.3x:Cooper & al. 1989‡	20.0x:Wahren , 1977†
	11.0x:Wahren & al. 1977†	1.5x: Stanley & al. 1988‡		23.0x: Wahren & al. 1971†
		2.0x: DeFronzo & al. 1981‡		
		2.3x: Jenkins & al. 1985‡		
		2.3x: Shilo & al. 1990‡		
		10.0x: Wahren, 1977†		
		12.0x: Ahlborg & al. 1974†		
		16.0x: Wahren & al. 1975†		
61-90	19.0x: Wahren, 1977†	1.7x: Jenkins & al. 1985‡		
		2.0x: Jenkins & al. 1986‡		
		16.0x: Ahlborg & al. 1974†		
91-120		2.0x:Wasserman & al. 1984‡		
121-150		2.5x: Knapik & al. 1988‡		
		15.0x: Ahlborg & al. 1974†		
≥151		11.0x: Ahlborg & al. 1974†		

*This table contains only data from 12-14 overnight fasted human studies. These data represent the increase in glucose uptake from the resting value reported in the same paper.

†Leg glucose uptake.

‡Glucose utilization i.e. whole body glucose uptake.

TABLE IX

Exercise and Metabolic Glucose Clearance*

AUTHORS	EXERCISE		RESULT
	Intensity (%VO_2 max)	Duration (min)	Δ increase from rest
Cooper et al., 1989	40	40	
	80	40	2.0X
Jenkins et al., 1985	60	60	1.9X
Stanley et al., 1988	43 ± 5	30	1.3X
		50	1.4x

*This table only contains data from 12-14 overnight fasted human studies.

TABLE X

Effects of Glucagon and Epinephrine on Hepatic Glucose Metabolism

	Glycogenolysis	Gluconeogenesis	Net Uptake of Glucogenetic Substrates	Fractional Extraction of Glucogenetic Substrates	Glucogenetic Efficiency	Splanchnic Delivery of Glucogenetic Substrates
Glucagon	Transient	Persistent	↑	↑	↑	—
Epinephrine	Transient	Persistent	↑	—	—	↑

* Based on the data from Refs. 12, 49, 56, and 57.

Adapted from: SACCÀ, L. Role of counterregulatory hormones in the regulation of hepatic glucose metabolism. *Diabetes/Metabolism Rev.* 3: 207-229, 1987.

CHAPTER 2

ROLE OF INSULIN AND GLUCAGON IN THE REGULATION OF HEPATIC GLUCOSE PRODUCTION DURING EXERCISE IN HEALTHY MALE SUBJECTS

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ABSTRACT

This experiment was conducted to further characterize the role of insulin and glucagon in the regulation of glucose production during 2 hours of mild to moderate exercise (40% VO_2 max) in postabsorptive healthy male subjects. Endogenous insulin and glucagon were suppressed by the infusion of somatostatin (SRIF) and the pancreatic hormones were replaced singly (SRIF + INS or SRIF + GLUC) or in combination (SRIF + INS + GLUC) to match the hormonal concentrations observed during exercise in control subjects. Glucose kinetics were determined by tracer methodology using the stable isotope, D[2,3,4,6,6-^{2}H]-glucose. Hepatic glucose production increased from 12.1 ± 0.4 at rest to 27.6 ± 3.0 μmol/kg•min during exercise. In the absence of glucagon, this increase in hepatic glucose production during exercise was totally abolished. When insulin was made deficient in the presence of glucagon there was an overshoot in the increase in hepatic glucose production during exercise to 36.4 ± 4.2 μmol/kg•min. The normal increase in hepatic glucose output was reproduced when both insulin and glucagon were replaced during exercise. From these observations, it is concluded that during prolonged mild to moderate exercise in postabsorptive healthy subjects the increase in hepatic glucose production is essentially dependent on the increase in glucagon. The presence of insulin, however, is important to prevent glucose overproduction and possibly hyperglycemia.

Key words: glucose output, stable isotope, exercise, pancreatic hormones, hormonal regulation, somatostatin.

INTRODUCTION

During prolonged exercise of moderate intensity, it has been shown that blood glucose remains relatively stable because hepatic glucose production increases to meet the demand by the working muscles (1-3). Human and animal studies support the concept that such glucose homeostasis is regulated by neuro-hormonal signals (1, 3-8). It has been well documented that exercise of moderate intensity is associated with a decrease in plasma insulin (9-11) as well as an increase in plasma glucagon (12-14) and in plasma catecholamines (15-16).

The increase plasma catecholamines during exercise results from the increased release by both the adrenal medulla and the sympathetic nerve endings throughout the body (6, 16-18). Such an increase in hormone concentration probably contributes to the stimulation of hepatic glucose production during exercise (19). Their role, however, is probably secondary since the increase in hepatic glucose production during exercise in man is not impaired by beta and alpha blockers (20). The rise in catecholamines is also responsible, at least in part, for insulin suppression through α-adrenergic stimulation (6-7, 9, 21-23). Although this drop in insulin concentration may not be essential for the increase in hepatic glucose production during exercise (24), evidence shows that the reduction in the pancreatic hormone enhances hepatic glucose output during exercise (17, 25-28).

Issekutz and Vranic (29) were the first to show in dog studies that the suppression of glucagon by somatostatin during exercise was associated with a decrease in hepatic glucose production resulting in hypoglycemia. Such a decrease in hepatic glucose output could be corrected by glucagon replacement and hypoglycemia prevented. A number of animal studies have since confirmed these observations thus establishing the primary role of glucagon in enhancing hepatic glucose production during exercise, at least in animal models (1, 25, 26, 29-32). In man, however, such a role for glucagon has been questioned. Both Wolfe et al. (34) and Shilo et al. (35) have studied the effect of

moderate intensity exercise in man during somatostatin infusion in combination with glucagon plus low and high doses of insulin replacement. They concluded that both the rise in glucagon and the drop in insulin are essential to ensure glucose homeostasis during exercise. On the other hand, Björkman et al. (36) could not show any impairment in the rise of hepatic glucose production during moderate intensity exercise in man when endogenous glucagon was suppressed by somatostatin infusion. From these observations, it was concluded that glucagon was not essential for glucose homeostasis during exercise.

The purpose of this study was to further characterize the role of glucagon and insulin in the regulation of the exercise-induced rise in hepatic glucose production in healthy man. Endogenous glucose production was measured by stable isotope methodology during mild to moderate intensity exercise while endogenous pancreatic hormones were suppressed by somatostatin and replaced singly or in combination by exogenous infusion. Our results support a preponderant role for glucagon in regulating the rise in hepatic glucose production during prolonged mild to moderate intensity exercise in healthy man. They also support an important role for insulin in restraining the glucagon stimulated increase in hepatic glucose production during exercise.

METHODS

Subjects

After an overnight fast (~ 15-hour postabsorptive), five (5) healthy male subjects, 26.1 ± 0.6 years of age, within 10% of their ideal body weight, participated in this study. All subjects were instructed to follow a well-balanced diet (55% carbohydrates, 30% lipids, and 15% proteins) and maintained a nutritional diary including daily physical activities over the week prior to the study. They were also instructed not to participate in any vigorous physical activities the day preceding the study. The last meal before the study was standardized and given at 18h00 the day before.

Every subject had a normal 2-hour OGTT, a normal history and physical exam. They also had normal ECG and chest x-ray. The complete blood count, biochemistry profile and liver function test were also normal. The protocol was approved by the ethic committee of the IRCM and a signed informed consent was obtained before each study.

Experimental Design

Altogether, twenty-four (24) experiments were performed. All studies were started at 8h00 AM. The exercise was performed on a bicycle ergometer at mild to moderate intensity (40% VO_2 max) over a 2-hour period (9h00-11h00). VO_2 max was evaluated during the week preceding the study with a direct open air circuit (Quinton) on a bicycle ergometer (Monark) using a graded protocol (37). Resting as well as exercise ECG were recorded to rule out cardiovascular disease.

During exercise, endogenous insulin and glucagon were suppressed by SRIF (kindly provided by Ferring Inc., Willowdale, Ontario) at an infusion rate of 0.1 µg/kg•min throughout the study. Insulin (Velosulin Human R, Nordisk Gentofte Canada Inc., Mississauga, Ontario) and glucagon (Lilly Research Laboratories, Indianapolis, Indiana) were then replaced singly or in combination (insulin at 0.05 mU/kg.min and glucagon at 1.0 ng/kg•min from 0 to 60 min and at 1.5 ng/kg•min from 60 to 120 min) so as to duplicate the levels of pancreatic hormones obtained in control experiments where saline

was infused during exercise. However, it is important to remember that portal glucagon levels are twice as high as peripheral levels and that portal glucagon levels are pivotal for the action of the hormone on hepatic metabolism. Thus, the aim of glucagon replacement was to reproduce portal levels. Because insulin acts mainly in the periphery, the aim of insulin replacement was to duplicate peripheral levels. Experiments were also done while subjects were resting during saline infusion. Blood samples were drawn every 10 minutes throughout the last hour of exercise.

The following protocols were performed: **protocol 1:** resting controls where saline was infused throughout the 3-hour study period with subjects seated; **protocol 2**: exercise control where saline was infused throughout the study without hormonal modification; **protocol 3**: exercise during insulin and glucagon deficiencies induced by SRIF infusion; **protocol 4**: exercise during glucagon deficiency induced by SRIF + insulin replacement; **protocol 5**: exercise during insulin deficiency induced by SRIF + glucagon replacement; **protocol 6**: exercise during SRIF + insulin and glucagon replacement for validation of the experimental model.

During protocols 3 and 4 where glucagon was made deficient, subjects were hooked up to the Biostator to prevent blood glucose to fall below 3.5 mmol/L during exercise. The Biostator was put on a dynamic program mode.

A first catheter (20-gauge teflon) was placed into a forearm vein for infusion of saline, D_5-glucose, SRIF, and pancreatic hormones and a second catheter (20-gauge) was placed into a hand vein and kept at 68°C to provide arterialized venous blood for sampling (38). The cannula for sampling was kept patent by a saline drip. All reagents were prepared on the morning of the experiment. Pyrogen free D_5-glucose, SRIF, insulin and glucagon were dissolved separately in 0.9% NaCl. Hormone preparations contained ~1 ml of the subject's own serum to minimize hormone adhesion to the tubing. In order to maintain an exercise intensity of 40% of VO_2 max at a cycling frequency of 50 RPM, the load on the bike was checked every five minutes. Blood pressure and heart rate were

registered at 30-minute intervals throughout the experiment. The exercise was performed in a temperature-controlled room set at 22°C for the subject's comfort.

Tracer Methods

Glucose turnover was measured using the isotope D-[2,3,4,6,6-$^2H_5$]-glucose (Merck Sharp and Dome, Pointe Claire, Québec). This glucose isotope was chosen because we wanted to measure both glucose turnover and gluconeogenesis simultaneously as described in a previous paper (39). Gluconeogenesis data are presented in the accompanying paper. The D-[2,3,4,6,6-$^2H_5$]-glucose was given as a prime-constant infusion; a bolus of 250 mg followed by a constant infusion at a rate of 3.0 mg/min. This dose was calculated to obtain an isotopic enrichment between 1 and 2%. The D-[2,3,4,6,6-$^2H_5$]-glucose was started at 8h00 AM, one hour before exercise and carried through the 2-hour exercise period (9h00 AM to 11h00 AM). Only data collected during the last hour was used for the calculation of glucose turnover, thus allowing two hours for isotope equilibration.

Laboratory Methods

D-d_5-glucose was separated from lactate and amino acids by column chromatography (40). The isotope was then measured by gas chromatography-mass spectrometry under electron impact in the mode of selected ion monitoring (Hewlett Packard model 5890 (GC) and 5970 (MS)). D-d_5-glucose was analyzed as its 6-acetyl-[1,2:3,5]-bis-butaneboronyl-α-D-glucofuranose derivative (41) and ions at *m/z* 297 and 302 were measured.

After deproteinization with 6% PCA, plasma glucose was measured by the hexokinase method (42). Plasma insulin and glucagon were measured by radioimmunoassay (Biodata, NCS Diagnostics Inc., Mississauga, Ontario). Cortisol was measured by radioimmunoassay using magnetizable particle and growth hormone by immunoradiometric assay using monoclonal antibody coated tubes (NCS Diagnostics Inc.).

Calculations

During exercise, the isotopic steady state was disrupted and, therefore, the non-steady-state equations of Steele (43) were used to calculate glucose kinetics based on the one compartment model of glucose kinetics. For calculations, the enrichment factor (*ef*) was used: $ef = R/(R+1)$ where $R = I_l / I_u$. I_l represents the intensities of (M-butyl)$^+$ ion at *m/z* corresponding to labeled glucose (*m/z* = 302), and I_u the intensities of (M-butyl)$^+$ ion at *m/z* corresponding to unlabeled glucose (*m/z* = 297). Each *R* value was obtained by direct measurement on the mass spectrometry and corrected by standard curves before using them in the formula, therefore, correcting for the isotopic purity of the tracer and the natural abundance contribution to the measured m/z ratios. Standard curves, obtained by weighing known amounts of labeled and unlabeled compounds were done for every new batch of tracer. The following equation for *Ra* was used:

$$Ra = \frac{(F)}{ef} - \frac{N(def)}{ef(dt)}$$

where *Ra* = the rate of appearance (μmol/kg•min); *F* = the rate of infusion of stable isotope (μmol/min); *N* = P•V•G where *P* = the pool fraction (0.65), *V* = the volume of distribution (ml), *G* = the blood glucose concentration (μmol/ml), *d* = the delta and, *t* = the time (minute). Rates of glucose appearance in the text and figures represent final values after subtraction of exogenous dextrose infusion from the Biostator.

Statistical Analysis

Data are expressed as means ± SEM. Because of the time required for equilibration of the tracer within the volume of distribution, only data from the second hour of exercise were used for analysis. Since all data measured were relatively stable over the second hour of exercise, we have chosen to express them as a mean over the exercise period. Therefore, for statistical purposes, the data presented here were computed for the last hour of the exercise period or from 60 to 120 minutes. Since all

subjects did not participate in all six protocols, each experiment is considered as a different subject for analytical purposes. Therefore, statistical comparisons were made using one-way analysis of variance (ANOVA) and differences between experimental groups were determined using *t*-test with Bonferroni correction for multiple testing using 0.05 level as statistical significance (44). However, analysis of variance (ANOVA) using repeated measures were also done for the three subjects who completed all six protocols. The level of significance was the same for both types of analysis.

RESULTS

Descriptive Data

The mean BMI for all subjects was 21.9 ± 0.3 kg/m^2 with a mean VO_2 max of 41.67 ± 0.85 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$. The analysis of their nutritional diaries confirmed that they followed the dietary instructions and maintained a mean caloric intake of 2474 ± 87 kcal (from 1539 to 3203) distributed as follows: $49.1 \pm 0.6\%$ carbohydrates, $19.0 \pm 0.4\%$ proteins and $32.0 \pm 0.7\%$ lipids. None of the subjects participated in vigorous exercise on the day before the experiment.

Pancreatic Hormones

During exercise under saline infusion (protocol 2), the plasma insulin concentration decreased to 70.4 ± 1.7 pmol/L compared to 98 ± 10 pmol/L in the resting control (Table I). When insulin was made deficient with (protocol 3) or without glucagon deficiency (protocol 5), SRIF infusion at 0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ suppressed plasma insulin to the lower limit of the assay at 37.3 ± 1.0 and 36.3 ± 0.2 pmol/L, respectively (these can not be distinguised from zero). When insulin was replaced in the absence (protocol 4) or presence of glucagon (protocol 6), insulin infused exogenously at 0.05 $mU \cdot kg^{-1} \cdot min^{-1}$ achieved plasma levels of 68.8 ± 2.3 and 73.6 ± 1.52 pmol/L, respectively, and both levels were significantly lower than those obtained at rest (protocol 1), but similar to those observed in the exercising controls (protocol 2).

During exercise under saline infusion (protocol 2), the plasma glucagon concentration increased from a resting value of 107.0 ± 2.9 to 127.3 ± 4.4 pg/ml, but did not reach statistical significance (Table II). When glucagon was made deficient by the SRIF infusion in the absence (protocol 2) or presence (protocol 4) of insulin, plasma glucagon levels were decreased to the lower limit of the assay at 40.8 ± 2.6 and 37.1 ± 4.3 pg/ml, respectively ($p < 0.05$). When glucagon was replaced at 1.0 and 1.5 $ng \cdot kg^{-1} \cdot min^{-1}$ (from 60 to 120 min) in the absence (protocol 5) or presence of insulin (protocol 6), peripheral levels of 229.9 ± 19.0 and 251.8 ± 1.0 pg/ml were obtained, respectively

($p < 0.05$). These levels are thus approximating the portal glucagon levels estimated from the control exercise conditions.

Plasma Glucose

Two hours of mild to moderate ergocycle exercise (40% VO_2 max) did not result in a significant decrease in plasma glucose (4.26 ± 0.13 mmol/L) (Table III). When glucagon was made deficient without (protocol 3) or with (protocol 4) insulin replacement, plasma glucose decreased slightly to 3.85 ± 0.10 and 3.74 ± 0.19 mmol/L, respectively. In these two protocols, plasma glucose was prevented from decreasing to hypoglycemic levels by the Biostator. Over the last hour of exercise, the Biostator infused glucose at a rate of 6.88 ± 1.15 and 7.52 ± 1.19 $\mu mol \cdot kg \cdot^{1} .min^{-1}$ in protocols 3 and 4, respectively indicating that glucagon plays a crucial role in maintaining plasma glucose during mild to moderate exercise. When insulin was made deficient in the presence of portal levels of glucagon (protocol 5), plasma glucose increased significantly to 6.69 ± 0.24 mmol/L (protocol 5), indicating that insulin plays an important role in preventing a rise in plasma glucose during exercise.

Hepatic Glucose Production (*Ra*)

During saline infusion (protocol 2) exercise was associated with a 2-fold increase in *Ra*, from 12.07 ± 0.43 to 27.56 ± 3.03 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. In the absence of glucagon with (protocol 4) or without (protocol 3) insulin replacement, endogenous glucose production remained at 13.02 ± 2.91 and 17.00 ± 2.10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ respectively and was not different from the resting control rates (protocol 1). These observations also support an important role for glucagon for the increase in hepatic glucose production during exercise. When insulin was made deficient in the presence of portal vein levels of glucagon (protocol 5), hepatic glucose production increased to three times above the resting level (36.35 ± 4.17 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $p < 0.05$)) indicating that basal insulin, even at lower levels during exercise, plays an important role in restraining the increase in hepatic glucose production during exercise and in preventing hyperglycemia. Finally, the

replacement of insulin and glucagon during SRIF infusion (protocol 6) reproduced the hepatic glucose production observed in the exercising control experiment (protocol 2) (29.30 ± 0.85 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) validating the model used in this study.

Plasma Cortisol and Growth Hormone

Plasma cortisol levels were 184 ± 7 nmol/L in the resting controls and increased significantly in all exercise protocols (389 ± 31 nmol/L; 291 to 529 nmol/L). Growth hormone levels also increased during exercise from resting levels of 6.72 ± 0.20 to 10.29 $\pm$ 0.41 mIU/L. During SRIF infusion, they were all suppressed to non detectable levels.

DISCUSSION

This study was designed to further characterize the role of glucagon and insulin in the regulation of hepatic glucose production during mild to moderate intensity exercise in healthy male subjects. We used somatostatin to suppress the endogenous secretion of pancreatic hormones and these were replaced singly or in combination. The present study demonstrates that glucagon is essential for the increase in hepatic glucose production during exercise at 40% VO_2 max. Our observations also support an important role for insulin, even at lower levels, in restraining the glucagon stimulated rise in hepatic glucose production induced by exercise.

In the exercising controls (protocol 2) in whom saline was infused, mild to moderate exercise resulted in a significant decrease in plasma insulin from 98.3 ± 10.0 to 70.7 ± 1.7 pmol/L and an increase in plasma glucagon from 107.0 ± 2.9 to 127.3 ± 4.4 pg/ml though the latter did not reach statistical significance (Tables I and II). The magnitude of the changes in the pancreatic hormones are similar to those reported by some investigators (3, 34, 35, 46), but different from those reported by others (36, 47, 48). It is possible that in the latter studies the variation in the duration of exercise could account for the different observations. It should also be pointed out that several other factors can influence the hormonal response to exercise such as the intensity of exercise, the level of physical fitness, the antecedent diet and the nutritional state (6). In our experimental conditions, mild to moderate intensity exercise (40% VO_2 max) for two hours was sufficient to induce a 26% decrease in insulin and a 20% increase in glucagon, hormonal changes that are quite consistent with other studies done under similar conditions.

Under these conditions, exercise was associated with a maintainance of plasma glucose (4.3 ± 0.13 mmol/L) and a 2-fold increase in hepatic glucose production from 12.1 ± 0.4 to 27.6 ± 3.0 μmol/kg•min ($p < 0.05$). These observations are also consistent with those made by other investigators (4, 35, 36, 46, 49).

Validation of our somatostatin model was dependent on the possibility of duplicating the hormonal concentrations observed in the exercising controls and more importantly of reproducing the metabolic changes induced by exercise. In protocol 6 where both pancreatic hormones were replaced, insulin infusion resulted in peripheral insulin levels of 73.6 ± 1.5 pmol/L, levels that are similar to those observed in the exercising controls (70.7 ± 1.7 pmol/L). These similar peripheral levels are achieved, however, at the expense of lower portal vein insulin (45). Glucagon, on the other hand, was infused at 1.0 ng/kg•min for the first hour of exercise and at 1.5 ng/kg•min for the second hour in an effort to achieve peripheral levels 1.5 to 2.0 times those observed in the exercising controls (protocol 2). These infusion rates resulted in peripheral levels of 251.8 ± 1.0 pg/ml compared to 127.3 ± 4.4 pg/ml in the exercising controls. This would be consistent with the portal peripheral gradient measured by others investigators (45, 50, 51). We are therefore quite satisfied that we reproduced the portal vein hormonal milieu observed during mild to moderate intensity exercise. But more importantly, we were able to reproduce the metabolic changes observed in the exercising controls. Plasma glucose concentration was identical to that observed in the saline controls during exercise. Furthermore, the increase in hepatic glucose production was twice that calculated for the resting controls and comparable to the values obtained for the exercising controls. We, therefore, feel confident that the replacement of pancreatic hormones during somatostatin infusion was adequate.

Somatostatin infusion resulted in adequate suppression of both plasma insulin and glucagon. Both hormones were suppressed to below the limit of detection of the assays (Tables I and II). The efficiency of somatostatin was also confirmed by the suppression of plasma growth hormone to undetectable levels.

In our study, when glucagon secretion was suppressed by somatostatin in the absence (protocol 3) or presence of insulin (protocol 4), the increase in hepatic glucose production induced by exercise was totally abolished (Fig. 1). This strongly supports a

primary role for glucagon in the exercise-induced rise in hepatic glucose output. This is further corroborated by the observation that replacement of glucagon in the presence of insulin resulted in restoration of the increase in hepatic glucose production during exercise (Fig. 1, protocol 6). This is consistent with animal studies where glucagon has been shown to play an important role in increasing hepatic glucose production during exercise (1, 25, 26, 29-32). It is different, however, from the study of Björkman et al. (36) who could not show any impairment in the exercise-induced rise in hepatic glucose output during somatostatin infusion. It is possible that in their study the total suppression of glucagon was not achieved or that counterregulatory hormones compensated for the glucagon deficiency. Recently, data collected in man (34, 35) are consistent with our observations and support a preponderant role for glucagon in the increase in hepatic glucose output during exercise.

Furthermore, when glucagon was suppressed, even in the absence of insulin (protocol 3), hypoglycemia had to be prevented by the infusion of dextrose by the Biostator at a rate of 6.9 ± 1.2 μmol/kg•min over the last hour of exercise. This is quite similar to the glucose infusion rate reported by Shilo et al. (35) to prevent hypoglycemia when glucagon was suppressed by somatostatin infusion during exercise. These observations indicate that glucagon is crucial to prevent hypoglycemia during exercise. Since glucose was infused peripherally by the Biostator to maintain normal blood sugar, glucose infusion *per se* could not have affected glucose production by the liver.

When insulin was made deficient in the presence of glucagon (protocol 5), the increase in hepatic glucose production during exercise was 32% higher than in the exercising controls (protocol 2) (Fig. 1). This indicates that the circulating insulin concentration during exercise, even at lower levels compared to resting controls, plays a role in regulating hepatic glucose production by restraining the glucagon stimulated increase in glucose output. Though, it is possible that we are underestimating the physiological effect of insulin on this process because we have used peripheral insulin

levels which are lower than normal portal levels during exercise as demonstrated for glucagon by Wasserman et al. (52). This would also support an important role for the decrease in insulin levels during exercise in the response of the liver to glucagon. This would be in line with the observations made by Wolfe et al. (34) and Shilo et al. (35) who concluded that both the increase in glucagon and the decrease in insulin were important for the increase in hepatic glucose production during exercise. On the other hand, when insulin was replaced during glucagon deficiency, the Biostator had to infuse dextrose at a rate of 7.5 ± 1.2 μmol/kg•min to prevent hypoglycemia, an infusion rate that was not different from that required when both insulin and glucagon were made deficient. This suggests that the rise in glucagon is more important than the decrease in insulin to prevent hypoglycemia during exercise.

Based on our observations, it becomes obvious that it is the interaction between the two pancreatic hormones, insulin and glucagon, that is responsible for the fine regulation of hepatic glucose production during exercise and therefore for glucose homeostasis (Fig. 1). We have shown that in the absence of pancreatic hormones hepatic glucose production during exercise remains at a rate which is similar to that observed in resting controls (13.02 ± 2.9 μmol/kg•min). Since hepatic glucose production in exercising controls (protocol 2) was 27.6 ± 1.4 μmol/kg•min, this indicates that during mild to moderate exercise, 45% of glucose production is independent of the pancreatic hormones. We have already shown in resting dogs (53-55) that 50% of basal hepatic glucose production was dependent on pancreatic glucagon. It is possible, therefore, that the lack of decrease in glucose production in the absence of glucagon during exercise could be due to a non-pancreatic exercise-induced factors, such as catecholamines, on hepatic glucose production. Glucagon would be responsible for 11.43 μmol/kg•min or 40% of the increased hepatic glucose production during exercise. Our estimations are similar to the values obtained by Wasserman et al. (31, 32). On the other hand, insulin is important in preventing an overshoot in the exercise-induced increase in hepatic glucose

production which could result in hyperglycemia. It accounts for at least a 22% suppression of the glucagon stimulated increase in hepatic glucose production.

From these experiments it is concluded that glucagon plays a preponderant role in the exercise-induced increase in hepatic glucose production. But the lower insulin levels observed during exercise are important in preventing an overproduction of glucose by the liver and the development of hyperglycemia. It is therefore the interaction between the two pancreatic hormones that precisely regulates the increase in hepatic glucose production during exercise.

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TABLE I

PLASMA INSULIN LEVELS UNDER THE VARIOUS PROTOCOLS

PROTOCOLS	REST	EXERCISE				
	1 Saline	2 Saline	3 SRIF	4 SRIF+INS	5 SRIF+GLUC	6 SRIF+ INS+GLUC
			INSULIN (pmol/L)			
Subjects						
1	118.7	73.8	40.2	68.9	37.3	76.4
2		65.9	37.2	71.9	36.0	
3	85.0	70.7	36.0	59.9	36.0	73.2
4	91.2	72.4	36.0	71.7	36.0	71.1
5				71.8	36.2	
Mean ± SEM	98.3±1.0[bcdef]	70.4±1.7[ace]	37.3±1.0[abdf]	68.8±2.3[acef]	36.3±0.2[abdf]	73.6±1.5[acef]

[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

TABLE II

PLASMA GLUCAGON LEVELS UNDER THE VARIOUS PROTOCOLS

PROTOCOLS	REST	EXERCISE				
	1 Saline	2 Saline	3 SRIF	4 SRIF+INS	5 SRIF+GLUC	6 SRIF+ INS+GLUC
			GLUCAGON (pg/ml)			
Subjects						
1	101.4	122.4	41.6	41.6	222.0	252.7
2		118.0	29.0	46.4	256.4	
3	110.9	137.7	46.9	43.6	221.6	249.8
4	108.6	131.3	31.0	41.3	281.0	252.9
5				31.0	168.4	
Mean ± SEM	107.0±2.9[cdef]	127.3±4.4[cdef]	37.1±4.3[abef]	40.8±2.6[abef]	229.9±19.0[abcd]	251.8±1.0[abcd]

[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC

TABLE III

PLASMA GLUCOSE CONCENTRATIONS UNDER THE VARIOUS PROTOCOLS

PROTOCOLS	REST	EXERCISE				
	1 Saline	2 Saline	3 SRIF	4 SRIF+INS	5 SRIF+GLUC	6 SRIF+ INS+GLUC
			PLASMA GLUCOSE (mmol/L)			
Subjects						
1	4.50	3.90	4.07	3.81	4.86	4.64
2		4.37	3.20	4.22	5.39	
3	4.24	4.53	3.94	3.68	8.56	3.53
4	4.65	4.23	3.74	3.62	7.24	4.71
5				3.90	7.38	
Mean ± SEM	4.46±0.12[e]	4.26±0.13[e]	3.74±0.19[e]	3.85±0.10[e]	6.69±0.68[abcdf]	4.29±0.38[e]

[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

TABLE IV

HEPATIC GLUCOSE PRODUCTION UNDER THE VARIOUS PROTOCOLS

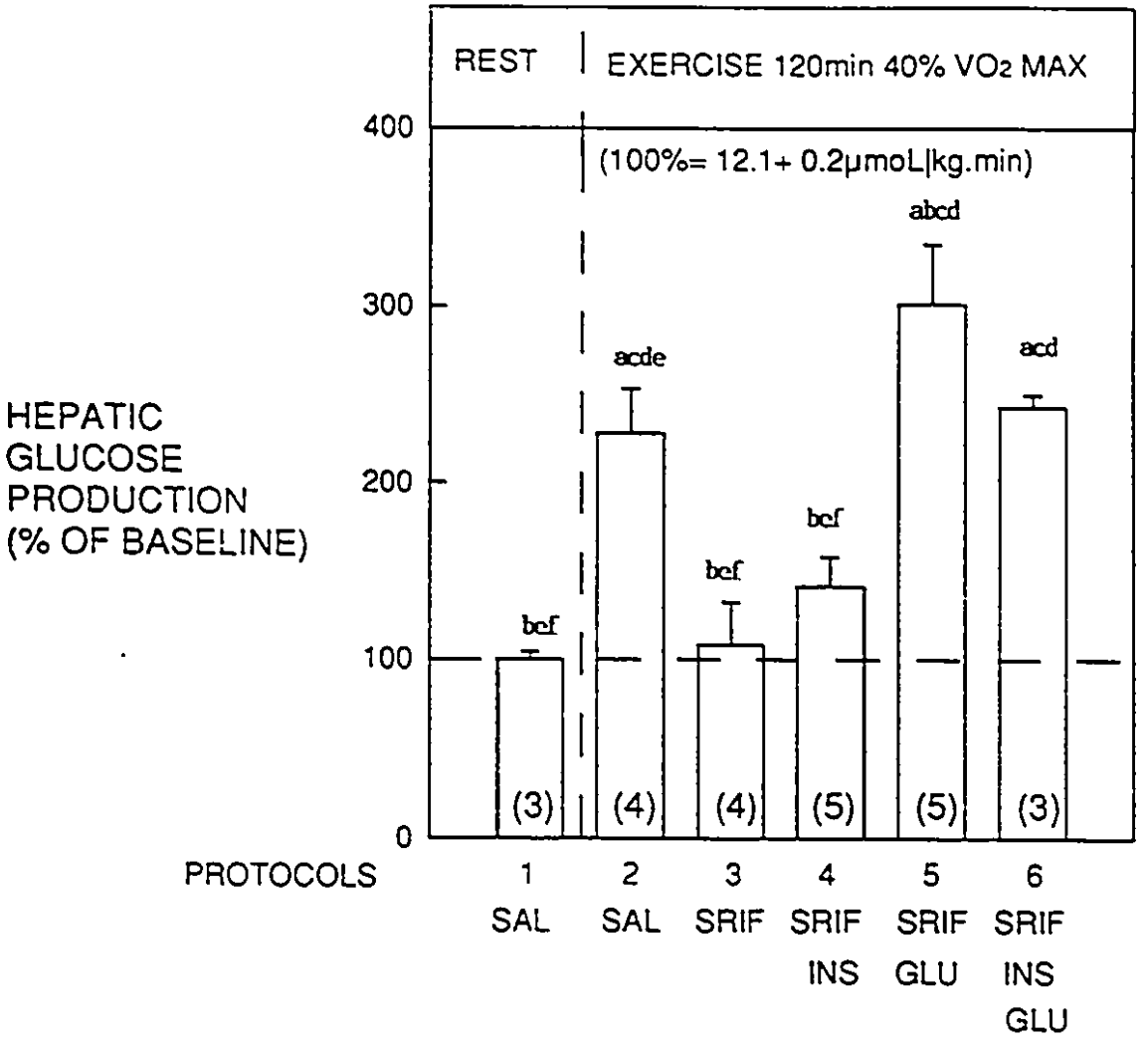
PROTOCOLS	REST	EXERCISE				
	1 Saline	2 Saline	3 SRIF	4 SRIF+INS	5 SRIF+GLUC	6 SRIF+ INS+GLUC
	HEPATIC GLUCOSE PRODUCTION ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)					
Subjects						
1	11.44	31.84	19.47	21.55	25.57	27.70
2		32.93	13.65	18.65	31.14	
3	11.89	19.89	5.34	10.44	46.52	29.57
4	12.89	25.56	13.62	13.85	45.76	30.61
5				20.49	32.76	
Mean ± SEM	12.07±0.43[bef]	27.56±3.03[acde]	13.02±2.91[bef]	17.00±2.10[bef]	36.35±4.17[abcd]	29.3±0.85[acd]

[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

FIGURE LEGEND

Figure 1

The effects of exercise at 40% VO_2 max on hepatic glucose production during the infusion of saline (protocol 2), SRIF (insulin and glucagon deficiencies - protocol 3), SRIF + insulin (glucagon deficiency - protocol 4), SRIF + glucagon (insulin deficiency - protocol 5), and SRIF + exogenous replacement of insulin and glucagon (protocol 6) compared to the resting state (protocol 1). The data are expressed as means ± SEM. a: Significantly different at $p < 0.05$ from resting control; b: significantly different at $p < 0.05$ from exercise control; c: significantly different at $p < 0.05$ from SRIF; d: significantly different at $p < 0.05$ from SRIF + INS; e: significantly different at $p < 0.05$ from SRIF + GLUC; f: significantly different at $p < 0.05$ from SRIF + INS + GLUC.



[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

FIGURE 1

CHAPTER 3

ROLE OF INSULIN AND GLUCAGON IN THE REGULATION OF GLUCONEOGENESIS DURING EXERCISE IN HEALTHY MALE SUBJECTS

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ABSTRACT

The present study was designed to further characterize the role of insulin and glucagon in the regulation of gluconeogenesis during 2 hours of mild to moderate intensity exercise (40% maximal oxygen consumption (VO_2 max)) in postabsorptive healthy male subjects. Endogenous insulin and glucagon were suppressed by somatostatin (SRIF) infusion and the pancreatic hormones were then replaced singly (SRIF + INS or SRIF + GLUC) or in combination (SRIF + INS + GLUC) to match the hormonal concentrations observed during similar exercise in control subjects. Rates of gluconeogenesis were estimated by the simultaneous infusion of D-[2,3,4,6,6-$^2H_5$]-glucose and L-[1,2,3-^{13}C]-alanine and measuring alanine conversion into glucose. SRIF infusion decreased insulin and glucagon to the lower limit of detection of the assay. Exercise increased gluconeogenesis by 44% above the resting level. When glucagon was made deficient, in the absence or presence of insulin, this increase in gluconeogenesis was totally abolished; in fact, under these circumstances, gluconeogenesis was decreased by 25% compared to resting controls. On the other hand, glucagon replacement during exercise in the absence of insulin resulted in a further increase in gluconeogenesis to 80% above resting value. Alanine turnover doubled during exercise and this independently of the hormonal milieu. It is concluded that during prolonged mild to moderate intensity exercise in postabsorptive healthy subjects, the exercise-induced glucagon increment is crucial for a proper gluconeogenic response to exertion. It is also suggested that the lower level of insulin during exercise still exert a restraining effect on glucagon stimulated gluconeogenesis.

KEY WORDS: Pancreatic hormones, human subjects, stable isotope, alanine turnover, gluconeogenesis, alanine.

INTRODUCTION

During prolonged exercise in the postabsorptive state, gluconeogenesis has been suggested to be an important contributor to hepatic glucose production in man (1). The importance of this contribution seems to be dependent on exercise intensity and duration (2, 3). Recently, it was shown that when gluconeogenesis was inhibited by blocking an enzymatic step of this metabolic process, running rats were not able to maintain euglycemia as well as the control animals (4).

Even though changes in gluconeogenic enzyme activities have been observed in exercising rats (5, 6), hormonal regulation of this process *in vivo* has not been completely elucidated. It has been shown repeatedly that during mild to moderate intensity exercise the concentration of glucagon increases and that of insulin decreases (2, 7-12). Much of what we know about hormonal regulation of gluconeogenesis comes from studies performed in dogs. In running dogs, Wasserman et al. (13, 14) have produced data showing that the exercise-induced rise in glucagon was important primarily for increasing the fractional extraction of alanine across the liver and, therefore, for enhancing the extraction of gluconeogenic precursors. This increase in glucagon induced by exercise is also associated with an increased intrahepatic conversion of alanine to glucose (15). The same group (14) reported that the exercise-induced fall in insulin accounted for an indirect glucagon-mediated effect on gluconeogenesis by enhancing the liver sensitivity to glucagon.

In the postabsorptive human subjects, the importance of gluconeogenesis during exercise is suggested by the increase in the hepatic uptake of gluconeogenic precursors (1-3, 16-20). However, because the fate of the extracted gluconeogenic precursors is multiple, and one can not equate hepatic uptake to gluconeogenesis. In human, the role of insulin and glucagon as mediators of the extraction of gluconeogenic precursors and of gluconeogenic rate are still poorly characterized. Therefore, our objective was to further investigate the role of insulin and glucagon on the regulation of gluconeogenesis during

prolonged mild to moderate exercise in healthy postabsorptive subjects. Our data indicate that the physiological increment in the level of glucagon during exercise is crucial for the increase in gluconeogenesis and also suggest that insulin, even at lower level, exerts a restraining effect on the glucagon stimulated gluconeogenesis.

METHODOLOGY

Subjects

Gluconeogenesis from alanine was measured concomitantly in the same subjects who participated in the glucose turnover experiments described in the accompanying paper.

Experimental Design

The subjects were submitted to a 2-hour ergocycle exercise of mild to moderate intensity (40% VO_2 max) from 9h00 to 11h00 AM. During exercise, endogenous insulin and glucagon were suppressed by SRIF (kindly provided by Ferring Inc., Willowdale, Ontario) at an infusion of 0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ throughout the study. Insulin (Velosulin Human R, Nordisk Gentofte Canada Inc., Mississauga, Ontario) and glucagon (Lilly Research Laboratories, Indianapolis, IN) were then replaced singly or in combination (insulin at 0.05 $mU \cdot kg^{-1} \cdot min^{-1}$ and glucagon at 1.0 $ng \cdot kg^{-1} \cdot min^{-1}$ from 0 to 60 minutes and at 1.5 $ng \cdot kg^{-1} \cdot min^{-1}$ from 60 to 120 minutes) so as to duplicate the levels of pancreatic hormones obtained in control experiments where saline was infused during exercise. However, since glucagon is mainly a liver hormone and since portal levels are twice that observed in the periphery, the aim of glucagon replacement was to reproduce hormonal levels observed in the portal vein. Because insulin acts mainly in the periphery, the aim of insulin replacement was to duplicate peripheral levels. Experiments were also done in resting subjects during saline infusion. Blood samples were drawn every 10 minutes throughout the last hour of exercise. The description of the various hormonal protocols is found in Table I and is described in details in the accompanying manuscript. In the text, protocol 2 is referred to as the exercise control condition whereas protocol 6 is referred to as the exercise model control condition.

During protocols 3 and 4 where glucagon was made deficient, subjects were hooked up to a Biostator to prevent blood glucose to fall below 3.5 mmol/L during exercise thus preventing the interference of counterregulatory hormones.

All studies were started at 8h00 in the morning. A catheter (20-gauge teflon) was inserted into a forearm vein for the infusion of saline, SRIF, pancreatic hormones, D_5-glucose, ^{13}C-alanine. Another catheter (20-gauge teflon) was placed into a hand vein and kept at 68°C to provide arterialized venous blood for sampling (21). The cannula for sampling was kept patent with a saline drip. All reagents were prepared on the morning of each experiment and pyrogene free D_5-glucose, ^{13}C-alanine, SRIF, insulin and glucagon were dissolved separately in 0.9% NaCl. Hormones preparations contained ~ 1 ml of the subject's own serum to minimize hormone adhesion to tubing. All infusions were standardized using Harvard Syringe pumps (Harvard Apparatus Co.). Total blood losses for each study was less than 250 ml.

Gluconeogenesis

Conversion of alanine to glucose was traced by a constant infusion of L-[1,2,3-^{13}C]-alanine (Merck Sharp and Dome, Pointe Claire, Québec, Canada) administered from 0 to 180 minutes. Gluconeogenesis was measured by double stable isotope technique using L-[1,2,3-^{13}C]-alanine as the tracer to follow the conversion of ^{13}C-alanine to ^{13}C-glucose and D-[2,3,4,6,6-$^{2}H_5$]-glucose to measure the production of ^{13}C-glucose (22). Alanine was chosen as the gluconeogenic precursor because it is the major glucogenic amino acid extracted by the liver, and constitutes with lactate the bulk of the gluconeogenic precursors (23-25). This choice was also based on the possible release of lactate by the liver under certain circumstances and on the possible removal of lactate through direct oxidation by skeletal muscles during exercise and recovery (26-28). ^{13}C-alanine was administered at a constant infusion rate of 4 mg/min resulting in a stable isotopic enrichment of 6 to 8% after 15 minutes of infusion, suggesting that an equilibrium has been reached within the alanine pool. This resulted in a stable ^{13}C-glucose enrichment of 0.5% after 30 minutes of the $^{13}C_3$-alanine infusion.

Exact infusion rates were determined for each experiment by measuring the isotope concentration in the infusate (D-[2,3,4,6,6-$^{2}H_5$]-glucose and L-[1,2,3-^{13}C]-

alanine) using mass spectrometry (Hewlett Packard model 5890 (GC) and 5970 (MS)) and by multiplying by the calibrated infusion rate.

Laboratory Methods

D-d_5-glucose and ^{13}C-alanine were separated from lactate and amino acids by column chromatography (29). D-d_5-glucose was measured as discussed in the accompanying paper. D-[1,2,3-$^{13}C_3$]-glucose was processed as D-d_5-glucose and measured at *m/z* 300. Samples for ^{13}C-lactate and ^{13}C-alanine were dissolved in methanol and analyzed by GC-MS as their bisterbutyldimethylsilyl-trifluoroacetamide derivatives. Ions at *m/z* 261, 264, 260 and 263 were monitored for each compound respectively. After deproteinization with 6% PCA, plasma glucose was analyzed by the hexokinase method (30). Serum lactate, alanine, and glycerol were measured enzymatically (31-33). Plasma for glucagon measurement was placed in prechilled tubes containing 50 µl of aprotinin Trasylol 10 000 K.I.U./ml (Miles no. 817113). Plasma insulin and glucagon were measured by radioimmunoassay (Biodata, NCS Diagnostics Inc.). Cortisol was measured with a radioimmunoassay using magnetizable particle (NCS Diagnostics Inc.).

Calculations

Gluconeogenesis is calculated by the method validated by Chiasson et al. (24) for steady and unsteady states conditions for radioactive isotopes. The formula for ^{13}C-glucose appearance is as follow:

$$Ra\ ^{13}C-glucose=(\frac{d^{13}C-glucose}{dt})\times P\bullet \cdot \quad Rd\times{}^{13}C-glucose$$

Gluconeogenesis is then calculated as follow:

$$\frac{Ra\ ^{13}C-glucose}{ef\ ^{13}C-alanine}$$

where *Rd* the rate of disappearance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$), *P* the pool fraction (0.65), *V* the volume of distribution (ml), *ef* the enrichment factor, *d* the delta and *t* the time (minute). Alanine turnover was calculated by dividing the rate of infusion of ^{13}C-alanine by the enrichment factor of ^{13}C-alanine from which we subtracted the rate of infusion ^{13}C-alanine.

Because of isotopic dilution in the hepatic oxaloacetate pool, this methodology provides an underestimation of the net rate of conversion of alanine to glucose (34). This underestimation has been calculated to be approximately two by Hetenyi et al. (35). Chiasson et al. (24) have already discussed the limitations of the technique. This technique does not allow us to quantify gluconeogenesis, but does allow to measure relative changes under various conditions. Therefore, the data generated by this method is used only as an index of gluconeogenesis. In this study, our results are expressed as a percentage of the resting value.

Statistical Analysis

Data are expressed as means ± SEM and the analysis of variance was utilized as discussed in the accompanying paper. The 0.05 level was chosen to indicate statistical significance.

RESULTS

Descriptive Data

The descriptive data of the subjects can be found in the accompanying paper.

Hormone Levels (Fig. 1)

The plasma insulin concentration decreased to 70.7 ± 1.7 pmol/L during exercise in the saline-treated control group (protocol 2) compared to 98.3 ± 10.0 pmol/L in the resting state (protocol 1). SRIF infusion without (protocol 3) or with glucagon replacement (protocol 5), resulted in suppressed plasma insulin to the lower limit of the assay. Exogenous insulin replacement in the absence (protocol 4) or presence (protocol 6) of glucagon resulted in plasma levels of 68.8 ± 2.3 and 73.6 ± 1.5 pmol/L, respectively both significantly lower than the resting values (protocol 1), and similar to those observed in the exercise control group (protocol 2).

The plasma glucagon concentration increased during exercise in the saline-treated group (protocol 2) from a resting value of 107.0 ± 2.9 ng/L to 127.3 ± 4.4 ng/L but did not reach statistical significance. When glucagon was made deficient by SRIF infusion in the absence (protocol 3) or presence of insulin (protocol 4), plasma glucagon levels were decreased to the lower level of the assay. When glucagon was replaced in the absence (protocol 5) or presence (protocol 6) of insulin, peripheral levels of 229.9 ± 19.0 and 251.8 ± 1.0 ng/L were achieved, respectively. These are consistent with portal vein levels of glucagon twice as high as those obtained in the peripheral circulation (36).

The plasma cortisol concentration increased with exercise from a resting value of 184 ± 7 to a mean value of 386 ± 38 nmol/L during exercise. The highest cortisol value was obtained during glucagon deficiency (476 ± 36 nmol/L) (protocol 4) while the lowest was found during insulin deficiency (253 ± 21 nmol/L) (protocol 5) but still remained 1.4 times higher than the resting value.

Plasma Glucose (Fig. 2)

Two hours of ergocyle exercise at 40% VO_2 max resulted in the maintain of euglycemic (4.26 ± 0.13 mmol/L) in the saline-treated group (Fig. 2). When glucagon was made deficient without (protocol 3) or with (protocol 4) insulin replacement, plasma glucose decreased to 3.74 ± 0.19 and 3.85 ± 0.10 mmol/L, respectively. In these two protocols, however, plasma glucose was prevented from decreasing to lower levels by the Biostator. In protocols 3 and 4, the biostator infused 6.88 ± 1.15 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and 7.52 ± 1.19 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ of dextrose, respectively. This suggests that glucagon plays a crucial role in maintaining plasma glucose during mild to moderate exercise. When insulin was made deficient in the presence of portal levels of glucagon (protocol 5), plasma glucose increased significantly to 6.69 ± 0.68 mmol/L suggesting that insulin plays an important role in preventing a rise in plasma glucose during exercise.

Gluconeogenic Precursors

The mean basal plasma alanine level was 0.227 ± 0.027 mmol/L. Although there were some variations in alanine concentration among the various hormonal protocols during exercise, none of them achieved significance (Table II).

The plasma lactate concentration increased with exercise from a resting value of 0.472 ± 0.005 to 0.999 ± 0.164 mmol/L in the control group (protocol 2, Table II). A similar rise was also observed in all other protocols with the lowest increase in protocol 5 (0.658 ± 0.082 mmol/L) where insulin was made deficient in the presence of glucagon.

Plasma glycerol concentration increased with exercise from a resting value of 0.035 ± 0.005 to 0.205 ± 0.014 mmol/L in the saline-treated control group (protocol 2, Table II). The increase in the plasma glycerol concentration was observed independently of the hormonal conditions. The lowest levels (0.109 ± 0.009 mmol/L) were observed when glucagon was made deficient in the presence of insulin (protocol 4).

Gluconeogenesis and Alanine Turnover (Fig. 3)

Gluconeogenesis, as estimated by the conversion of alanine to glucose, is shown in Table III expressed in absolute values ($\mu mol \cdot kg^{-1} \cdot min^{-1}$), and in Figure 3 as percent change from resting values. Gluconeogenesis increased with exercise from a resting value of 0.663 ± 0.040 to 0.959 ± 0.116 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in the saline-treated group (protocol 2). When glucagon was made deficient without (protocol 3) or with insulin replacement (protocol 4), gluconeogenesis remained below the resting value (0.491 ± 0.021 and 0.481 ± 0.032 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively). When insulin was deficient in the presence of glucagon (protocol 5), gluconeogenesis increased to a maximal value of 1.199 ± 0.205 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, representing an 80% increase above resting value. Finally, the gluconeogenic rate obtained in the exercise model-control protocol (protocol 6) was similar to the rate measured in the exercise control experiment (protocol 2).

Alanine turnover increased 2-fold during exercise independently of the hormonal milieu (Table III) reaching levels $219 \pm 14\%$, $186 \pm 33\%$, $174 \pm 19\%$, $207 \pm 8\%$, $204 \pm 10\%$ of the resting value in protocols 2, 3, 4, 5 and 6, respectively. All were significantly different from the resting state.

DISCUSSION

The purpose of this study was to evaluate the hormonal regulation of gluconeogenesis in healthy subjects during prolonged submaximal exercise. To do so, we used somatostatin as a tool to suppress the endogenous secretion of the pancreatic hormones while these were replaced singly or in combination. With this approach, we were able to assess the specific role of the exercise-induced rise in glucagon and fall in insulin levels. The results of the present investigation demonstrate for the first time in postabsorptive healthy subjects, the crucial role of glucagon in the stimulation of gluconeogenesis during exercise of mild to moderate intensity.

Our exercise protocol induced a 26% decrease in insulin ($p < 0.05$) and a 20% increase in glucagon levels ($p = \text{NS}$) (Fig. 1). The magnitude of these changes in pancreatic hormones achieved during exercise in the saline-treated control subjects were similar to those reported by most investigators (7, 9, 12, 37), though others have found different results (38, 39). It is possible that in the latter studies the variation in the duration and intensity of exercise could account for the different observations. It should be pointed out that several other factors, such as the level of physical fitness, the antecedent diet, and the nutritional state can also influence the hormonal response to exercise (40).

In the presence of SRIF, glucagon and insulin decreased to the lowest levels detectable by the assay, reflecting an adequate inhibition of the pancreatic hormones by somatostatin. Validation of our somatostatin model relied on the possibility of duplicating the hormonal concentrations observed during exercise in the saline-treated control group (protocol 2) and, more importantly, of reproducing the metabolic changes induced by exercise (protocol 6).

The glucagon concentrations measured in protocols 5 and 6 were twice as high as those observed in protocol 2; this difference was intentional. It has been shown by Blackard et al. (36) that glucagon levels in the portal vein are twice as high as the

peripheral levels. Since glucagon acts mainly on the liver, the aim of glucagon replacement was to reproduce portal vein levels peripherally. The estimated portal to peripheral gradient in protocols 5 and 6 were similar to those reported by others (41, 42). On the other hand, since insulin acts mainly in the periphery, the aim of insulin replacement was to duplicate peripheral levels. In protocol 6, where both pancreatic hormones were replaced, insulin infusion resulted in peripheral insulin levels that were close to those observed in the exercise saline-treated control group. These peripheral levels were achieved, however, at the expense of slightly lower portal vein insulin (43). But the strongest argument that we were able to duplicate the physiologic hormonal environment induced by exercise is the fact that we were able to reproduce in protocol 6 (SRIF + INS + GLUC) the changes in plasma glucose, in gluconeogenic precursors and in gluconeogenic rate observed in the exercise saline-treated control group (protocol 2). Thus we feel confident that our SRIF model was appropriate to study the effects of pancreatic hormones on gluconeogenesis.

In our study, mild to moderate intensity exercise was associated with a 45% increase in gluconeogenesis (Fig. 3; protocol 2). This is in good agreement with previous studies looking at uptake of gluconeogenic precursors by the splanchnic bed (1-3, 16-20) as well as the study by Stanley et al. (27) using radioactive tracer. These observations would support an important role for gluconeogenesis in maintaining glucose homeostasis during exercise. It can be calculated from our data that at least 4% of the hepatic glucose production was derived from alanine during exercise. Since alanine is diluted in the OAA pool, this provides an underestimation of net gluconeogenesis from alanine. If we use the correcting factor of 2.2 proposed by Hetenyi (1986), this would suggest a contribution of gluconeogenesis from alanine to total hepatic glucose production of 9%. If we add to this the possible contribution of pyruvate, lactate and glycerol, we can estimate a total possible contribution of gluconeogenesis to hepatic glucose production of approximately 35% during exercise. This is further supported by Wasserman et al. (13)

in dog studies where they have shown that gluconeogenesis played a crucial role in preventing hypoglycemia during exercise. In our protocols 3 and 4 (Fig. 3) where glucagon was made deficient during exercise, there was a complete inhibition of the exercise-induced rise in gluconeogenesis from alanine. This indicate that the presence of glucagon is essential for the rise in gluconeogenesis associated with exercise. This would be in agreement with data from dog studies published by other investigators (10, 13, 44). The presence of basal insulin in the absence of glucagon did not further suppress gluconeogenesis. This is not surprising since we have already shown that basal gluconeogenesis requires relatively high levels of insulin for suppression (45, 46, 49). However, when insulin was made deficient in the presence of glucagon (Fig. 3; protocol 5), there was a more pronounced increase in gluconeogenesis (80%) compared to the saline-treated control group (45%) during exercise. This suggest that the presence of insulin, even at its lower level during exercise, is still important in preventing an overexcessive increase in gluconeogenesis which could eventually lead to hyperglycemia. Thus, our data support an important role for gluconeogenesis in glucose homestasis during exercise. The exercise-induced increase in gluconeogenesis is finely regulated by the interaction of glucagon and insulin. Glucagon is essential for the rise in gluconeogenesis during exercise while insulin exert a restraining effect to prevent hyperglycemia.

Under our experimental conditions, alanine concentration was not modified by prolonged submaximal exercise or by the presence or absence of insulin or glucagon. These results agree with those of most investigators (16, 38, 50) except those reported by Björkman et al. (39), who showed an increase in alanine concentration during exercise. These differences are most likely due to different experimental conditions. Since alanine is primarily released by the contracting muscles (25, 51, 52) and since both exercise and glucagon increase alanine hepatic uptake (15, 53), it is not surprising that minor differences in the rate of appearance and disappearance could induce changes in alanine

concentration. In our study, the lack of changes in alanine levels during exercise suggest that the changes in the appearance and disappearance of alanine were the same. The total alanine turnover rate in the resting control group was similar to those reported by other investigators (35, 54, 55). During exercise, alanine turnover rate doubled independently of the hormonal milieu (Table III). This suggests that under those conditions, exercise *per se* is the major regulator in the flow of alanine from the muscle to the liver where it can be used in part as a substrate for gluconeogenesis. Alanine can also be used for protein synthesis and to a smaller extent oxidation in the Krebs cycle.

Lactate concentration increased 2-fold during exercise in all five protocols (Table II). The magnitude of the changes in lactate levels was similar to those reported during exercise of the same duration and intensity (9). As for alanine, these observations suggest that the muscle contraction *per se* is the major regulator of lactate release into the circulation. Glycerol levels were also increased with exercise as shown by other investigators (16, 38) indicating an increase in lipolysis. The increase in lipolysis was slightly blunted during exercise when insulin was replaced during glucagon deficiency (protocol 4), but not when both insulin and glucagon were made deficient. This suggests that lipolysis during exercise is regulated, at least in part, by the interaction insulin-glucagon, insulin inhibiting and glucagon enhancing triglycerides breakdown (40, 56).

Since alanine is the most important gluconeogenic amino acid (23-25), the measurement of alanine to glucose (15, 27) gives a good assessment of the changes in the gluconeogenic process. However, the isotopic dilution of our labeled precursor within the oxaloacetate (OAA) pool results in underestimation of the rate of gluconeogenesis. OAA is an intermediate common to both the gluconeogenic and oxidative pathways in hepatocytes. Consequently, some of the labeled carbons of the infused glucose precursor are lost as $^{13}CO_2$ and are replaced by unlabeled carbons originating from acetyl-CoA leading to a reduction in the enrichment of the carbon pool before conversion to glucose thus leading to an underestimation of gluconeogenesis from labeled alanine (34, 54, 57-

59). However, since the increased production of acetyl-CoA units from an increase FFA oxidation is matched by an increased hepatic uptake of gluconeogenic precursors as well as an increased activity of pyruvate carboxylase (5, 6, 13), neither the dilution of the OAA pool nor the dilution factor are changed significantly during prolonged exercise (13). Therefore, though this method can not be used to quantify gluconeogenesis, it is still a valid approach to measure changes in this process under various conditions. In this study, it is used as an index of gluconeogenesis to evaluate the role of insulin and glucagon in the regulation of gluconeogenesis during mild to moderate intensity exercise.

Based on *in vitro* and *in situ* studies, it is likely that the elevated glucagon/insulin ratio during exercise results in activation of adenyl cyclase in the hepatocyte. This will induce an increase in hepatic cAMP resulting in decrease in hepatic fructose-2,6-bisphosphate which is the major allosteric regulator of PFK-1, the rate-limiting enzyme of glycolysis. Thus a decrease in fructose-2,6-bisphosphate will favor the metabolic flux in the direction of gluconeogenesis (60). Winder and others have confirmed that the exercise-induced rise in plasma glucagon and the drop in plasma insulin were both involved in causing the decline in fructose-2,6-bisphosphate (6, 60, 61). Furthermore, Wassermar. et al. (10) have postulated from dog studies that glucagon increases gluconeogenesis through an increase in alanine extraction by the liver, but more importantly, through gluconeogenic efficiency within the hepatocyte (10). Our data are compatible with these observations. We have shown that alanine turnover was increased during exercise independently of the hormonal environment. Since the plasma alanine concentrations did not change, it indicates that hepatic uptake was increased in all protocol. Yet only the protocol with increase glucagon was associated with an increase in the conversion of alanine to glucose. Thus, the effect of glucagon on gluconeogenesis during exercise is mainly through an increased efficiency in the conversion of alanine to glucose.

Our data are the first to describe the fine regulation of hepatic gluconeogenesis during exercise in healthy postabsorptive male subjects through the interaction of glucagon and insulin. Our data support an important role for gluconeogenesis in maintaining glucose homeostasis during exercise. We have shown that glucagon is essential for the exercise-induced rise in gluconeogenesis during mild to moderate intensity exercise. On the other hand, insulin, even at lower levels, still play an important role in restraining the glucagon effect on gluconeogenesis. Therefore, the fine regulation of gluconeogenesis during exercise is dependent on the interaction between the two pancreatic hormones.

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TABLE I

HORMONAL PROTOCOLS

	REST	EXERCISE				
PROTOCOLS	1 Saline (3)	2 Saline (4)	3 SRIF (4)	4 SRIF+INS (5)	5 SRIF+GLUC (5)	6 SRIF+ INS+GLUC (3)
Saline	X	X				
SRIF			X	X	X	X
Insulin				X		X
Glucagon					X	X

SRIF: 0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$
Insulin: 0.05 $mU \cdot kg^{-1} \cdot min^{-1}$
Glucagon: 0 to 60 minutes: 1.0 $ng \cdot kg^{-1} \cdot min^{-1}$
60 to 120 minutes: 1.5 $ng \cdot kg^{-1} \cdot min^{-1}$

TABLE II

PLASMA GLUCONEOGENIC PRECURSORS CONCENTRATIONS

PROTOCOLS	REST	EXERCISE				
	1 Saline	2 Saline	3 SRIF	4 SRIF+INS	5 SRIF+GLUC	6 SRIF+ INS+GLUC
			LACTATE (mmol/L)			
Subjects						
1	0.470	1.28	1.22	1.18	0.694	0.939
2		1.19	1.33	0.777	0.582	
3	0.472	0.546	0.628	0.757	0.955	0.804
4	0.455	0.982	0.690	0.816	0.580	0.833
5				0.757	0.478	
Mean ± SEM	0.470±0.005[b]	0.999±0.164[a]	0.967±0.180[a]	0.857±0.081	0.658±0.082[b]	0.859±0.041
			ALANINE (mmol/L)			
Subjects						
1	0.176	0.267	0.401	0.330	0.303	0.393
2		0.253	0.243	0.350	0.207	
3	0.238	0.222	0.268	0.270	0.165	0.189
4	0.268	0.232	0.262	0.197	0.185	0.252
5				0.200	0.281	
Mean ± SEM	0.227±0.027	0.243±0.010	0.293±0.036	0.269±0.032	0.228±0.027	0.278±0.060
			GLYCEROL (mmol/L)			
Subjects						
1	0.035	0.205	0.127	0.117	0.173	0.147
2		0.211	0.280	0.089	0.143	
3	0.043	0.169	0.184	0.102	0.188	0.172
4	0.027	0.237	0.173	0.097	0.200	0.274
5				0.138	0.236	
Mean ± SEM	0.035±0.005[d]	0.205±0.014[ad]	0.191±0.032[ad]	0.109±0.009[a]	0.188±0.015[ad]	0.198±0.039[ad]

[a]Significantly different at $p < 0.05$ from resting control; [b]Significantly different at $p < 0.05$ from exercise control; [c]Significantly different at $p < 0.05$ from SRIF

TABLE III

GLUCONEOGENESIS AND ALANINE TURNOVER

PROTOCOLS	REST	EXERCISE				
	1 Saline	2 Saline	3 SRIF	4 SRIF+INS	5 SRIF+GLUC	6 SRIF+ INS+GLUC
		GLUCONEOGENESIS ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)				
Subjects						
1	0.656	0.867	0.495	0.437	0.890	1.093
2						
3	0.736	0.794	0.545	0.474	1.746	0.986
4	0.596	0.870	0.446	0.57[d]	0.877	1.069
5		1.304	0.480	0.440	1.282	
Mean ± SEM	0.663±0.040[e]	0.959±0.116[a]	0.491±0.021[e]	0.481±0.032[e]	1.199±0.205[acd]	1.049±0.033
		ALANINE TURNOVER ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)				
Subjects						
1	3.80	7.812	9.220	7.868	6.712	6.535
2						
3	2.702	6.782	7.756	5.138	7.301	7.547
4	4.178	9.148	3.859	5.138	7.301	7.641
5		7.459	5.649	6.764	8.054	
Mean ± SEM	3.56±0.44	7.80±0.50[a]	6.62±1.18[a]	6.18±0.69[a]	7.37±0.27[ac]	7.24±0.35[a]

[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF

FIGURE LEGEND

Figure 1

The effects of exercise at 40% VO_2 max on plasma insulin and glucagon concentrations during the infusion of saline (protocol 2), SRIF (insulin and glucagon deficiencies - protocol 3), SRIF + insulin (glucagon deficiency - protocol 4), SRIF + glucagon (insulin deficiency - protocol 5), and SRIF + exogenous replacement of insulin and glucagon (protocol 6) compared to the resting state (protocol 1). The data are expressed as means ± SEM. a: Significantly different at $p < 0.05$ from resting control; b: significantly different at $p < 0.05$ from exercise control; c: significantly different at $p < 0.05$ from SRIF; d: significantly different at $p < 0.05$ from SRIF + INS; e: significantly different at $p < 0.05$ from SRIF + GLUC; f: significantly different at $p < 0.05$ from SRIF + INS + GLUC.

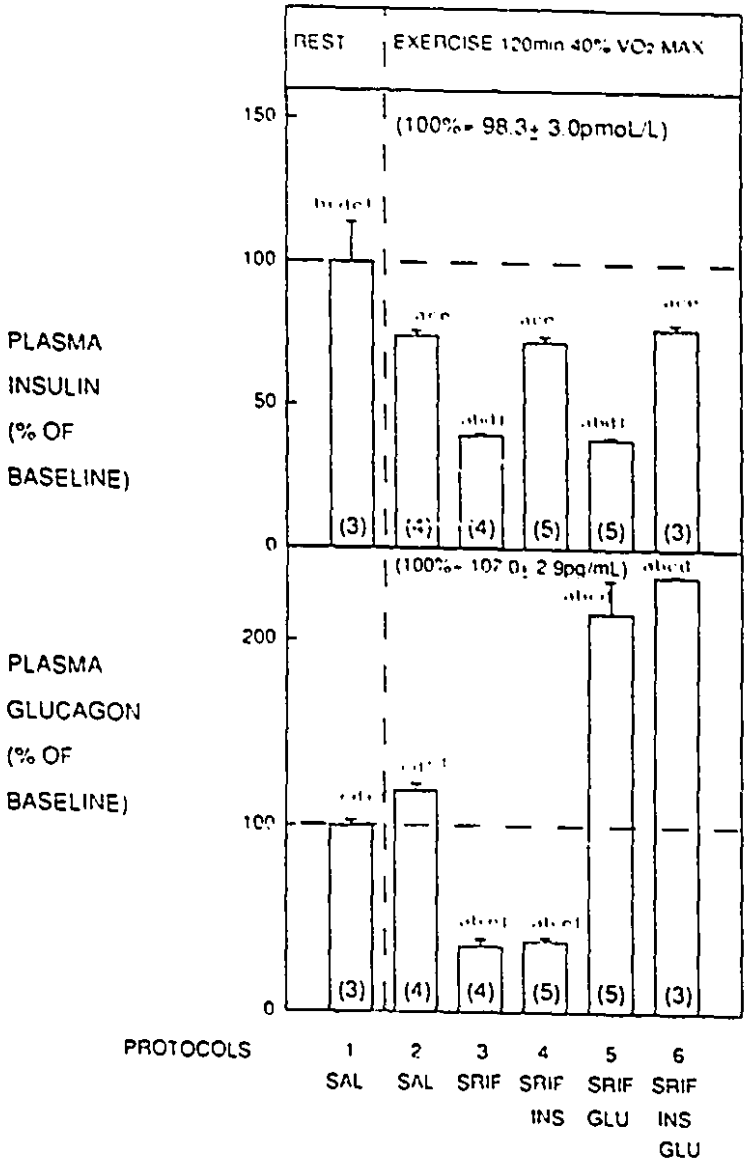
Figure 2

The effects of exercise at 40% VO_2 max on plasma glucose during the infusion of saline (protocol 2), SRIF (insulin and glucagon deficiencies - protocol 3), SRIF + insulin (glucagon deficiency - protocol 4), SRIF + glucagon (insulin deficiency - protocol 5), and SRIF + exogenous replacement of insulin and glucagon (protocol 6) compared to the resting state (protocol 1). The data are expressed as means ± SEM. a: Significantly different at $p < 0.05$ from resting control; b: significantly different at $p < 0.05$ from exercise control; c: significantly different at $p < 0.05$ from SRIF; d: significantly different at $p < 0.05$ from SRIF + INS; e: significantly different at $p < 0.05$ from SRIF + GLUC; f: significantly different at $p < 0.05$ from SRIF + INS + GLUC.

Figure 3

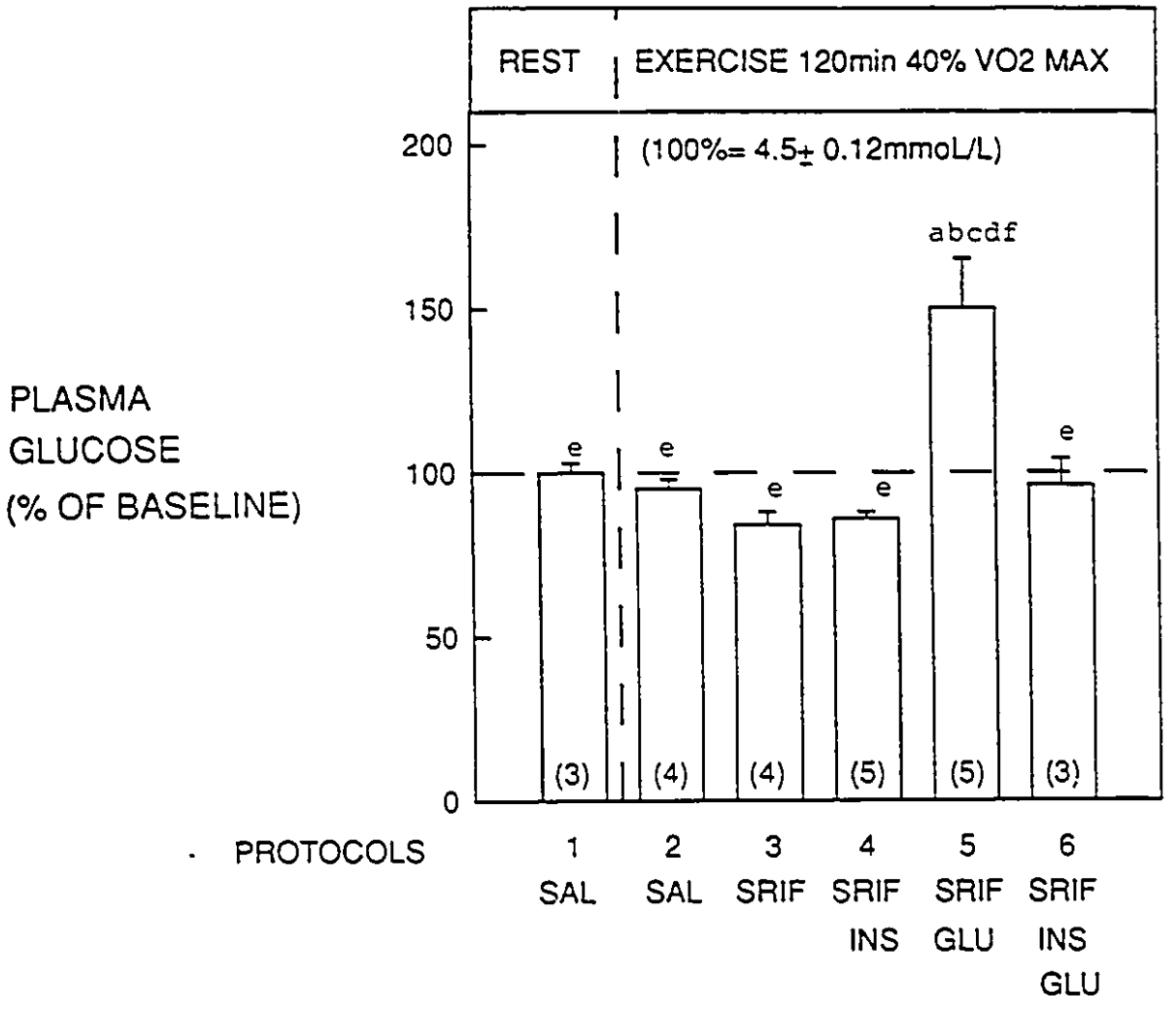
The effects of exercise at 40% VO_2 max on gluconeogenesis during the infusion of saline (protocol 2), SRIF (insulin and glucagon deficiencies - protocol 3), SRIF +

insulin (glucagon deficiency - protocol 4), SRIF + glucagon (insulin deficiency - protocol 5), and SRIF + exogenous replacement of insulin and glucagon (protocol 6) compared to the resting state (protocol 1). The data are expressed as means ± SEM. a: Significantly different at $p < 0.05$ from resting control; b: significantly different at $p < 0.05$ from exercise control; c: significantly different at $p < 0.05$ from SRIF; d: significantly different at $p < 0.05$ from SRIF + INS; e: significantly different at $p < 0.05$ from SRIF + GLUC; f: significantly different at $p < 0.05$ from SRIF + INS + GLUC.



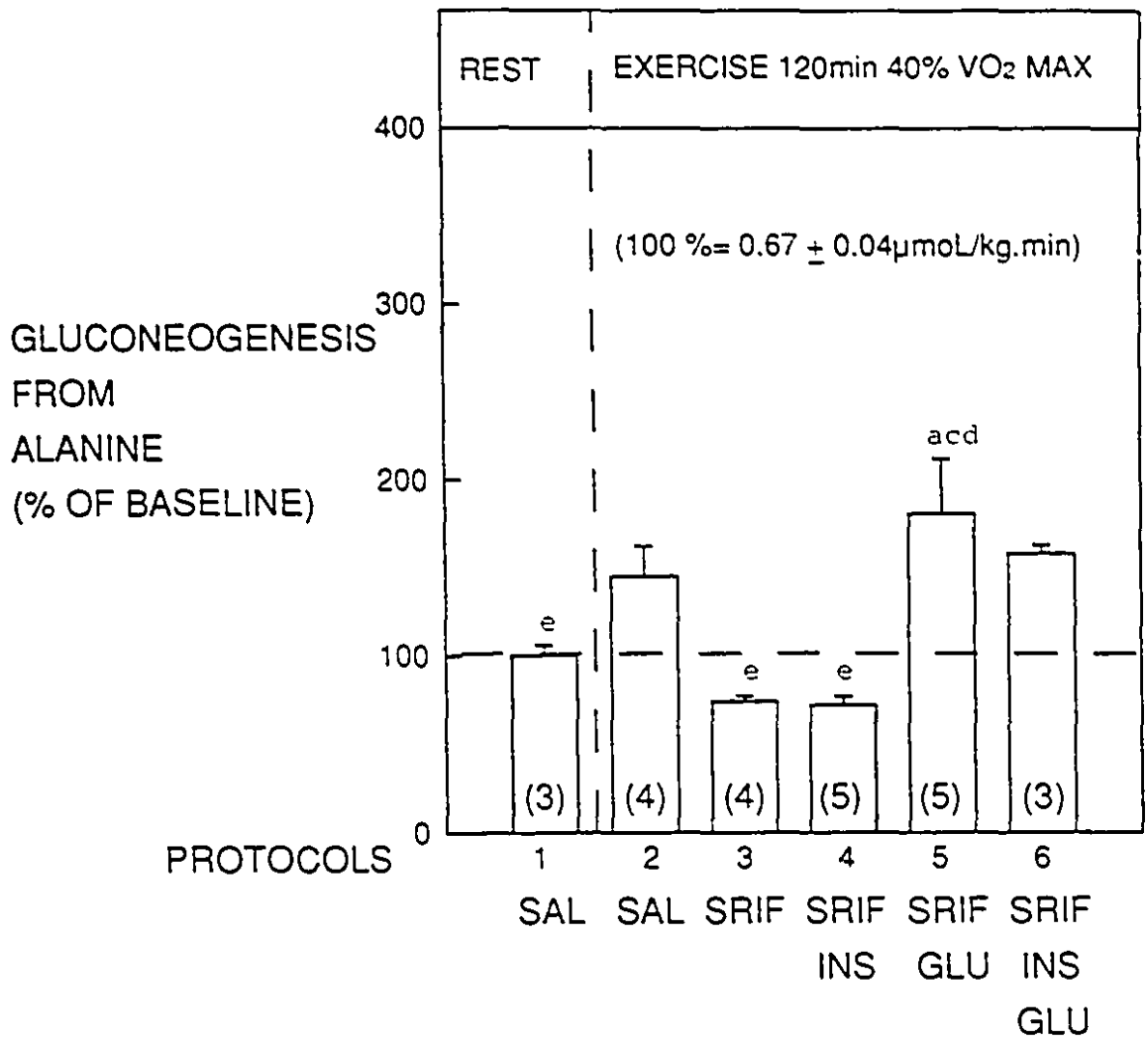
[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

FIGURE 1



[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

FIGURE 2



[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

FIGURE 3

CHAPTER 4

ROLE OF INSULIN IN THE REGULATION OF GLUCOSE UTILIZATION DURING EXERCISE IN HEALTHY MALE SUBJECTS

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Running title: Insulin regulation of glucose utilization during exercise.

Key words: Exercise, glucose utilization, insulin, FFA, glucose clearance rate, man

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ABSTRACT

The present study was conducted to further characterize the role of insulin in the regulation of glucose utilization during a 2-hour exercise at 40% VO_2 max in postabsorptive healthy male subjects. Endogenous insulin and glucagon were suppressed by somatostatin (SRIF) infusion and the pancreatic hormones were then replaced singly [SRIF+INS or SRIF+GLUC] or in combination [SRIF+INS+GLUC] to match the hormonal concentrations observed during similar exercise in saline-treated control subjects. Glucose kinetics were determined by tracer methodology using D-[2,3,4,6,6-^{2}H]-glucose. In all hormonal conditions, glucose utilization increased during 2 hours of exercise compared to resting controls. During exercise in the saline-treated controls, plasma glucose was maintained showing that glucose production equals glucose utilization. When glucagon was made deficient in the presence or absence of insulin it decreased further (25%) but was prevented to reach hypoglycemic levels by the Biostator. When glucagon was present in the absence of insulin, the plasma glucose increased by 50%. When insulin was present with or without glucagon, glucose utilization increased slightly over 2-fold. In the absence of insulin and glucagon it increased only by 50% while in the presence of glucagon without insulin it increased 3-fold. However, when correction was made for plasma glucose concentration and expressed as metabolic clearance, it increased 3-fold during exercise when insulin was present and only 2-fold when insulin was made deficient. It is concluded that in postabsorptive healthy subjects muscle contraction *per se* can stimulate glucose uptake by the exercising muscles even when insulin is made deficient. Insulin is important, however, for maximal glucose uptake by contracting muscles during prolonged mild to moderate exercise.

INTRODUCTION

Regulation of glucose uptake during exercise is multifactorial and hormones and metabolites can act together or in opposition to ensure precise modulation of glucose utilization. During prolonged mild to moderate intensity exercise, it has been shown that blood glucose remains relatively stable because hepatic glucose production increases to meet the demand of the working muscles (1-4). Exercise has been shown to increase glucose uptake by working muscle despite a decrease in insulin levels, indicating that glucose utilization by skeletal muscle is not directly related to the concentration of insulin during muscle contractions (5, 7-11, 14). Some studies, however, do support an important role for insulin in regulating glucose uptake by the contracting muscle (6, 12, 13, 16). Thus, the exact role of insulin in muscle glucose uptake during exercise is still unclear.

It has been shown *in vitro* that insulin was not required for the stimulation of glucose uptake by contracting muscles (8). On the other hand, Vranic and colleagues (12, 13) using depancreatized dogs maintained on constant intraportal basal insulin infusion during exercise found that small amounts of insulin were essential to allow adequate glucose uptake in the working muscles. In exercising dogs, Wasserman et al. showed that when insulin was clamped at resting level to prevent its normal decrease, there was a slight but inconsistent increase in glucose uptake (14). These authors concluded that since peripheral glucose uptake is affected by minute concentrations of insulin, the exercise-induced decrease in plasma insulin levels should not affect glucose uptake by the working muscles. It has been proposed that the increase in blood flow in the working limb (15) could compensate for the decrease in insulin concentration (16) by allowing increased insulin delivery.

Furthermore, the decline in insulin concentration together with rising catecholamine levels could contribute to the stimulation of lipolysis from adipose tissue

resulting in high FFA levels (17-19). Such an increase in FFA could result in decrease glucose uptake by the skeletal muscles through glucose-fatty acid cycle (20).

In vivo, Wasserman and his colleagues (21) recently presented human data suggesting that insulin and exercise worked synergistically to increase glucose uptake and this would be compatible with earlier observations made by DeFronzo et al. (16). However, such conclusions could be drawn only by extrapolating to a theoretical zero insulin concentration (21).

Although a few studies are available on the effect of muscle contractions *per se* on muscle glucose uptake *in vitro* (8, 22, 23) the precise role of the interaction between muscle contractions and insulin *in vivo* remains unclear. In the presently study, we have studied in healthy subjects the role of insulin in the regulation of glucose utilization during exercise. Glucose utilization and glucose metabolic clearance rate were measured using stable isotope methodology during mild to moderate intensity exercise while pancreatic hormones were suppressed by somatostatin and replaced singly or in combination by exogenous infusion. From our results, we conclude that muscle contraction *per se* can stimulate glucose uptake by the exercising muscle even when insulin is made deficient. However, the present of insulin is required for maximal glucose uptake by the contracting muscle.

RESEARCH DESIGN AND METHODS

Subjects

After an overnight fast (~15-hour postabsorptive), five (5) healthy male subjects, 26.1 ± 0.6 years of age, within 10% of their ideal body weight, participated in this study. They all had a normal 2-hour OGTT, a normal history and physical exam. They also had normal ECG and chest X-ray. The complete blood count, biochemistry profile and liver function test were normal.

All subjects were instructed to follow a well-balanced diet (50% carbohydrates, 35% lipids, and 15% proteins) and maintained a nutritional diary including daily physical activities over the week prior the study. They were also instructed not to participate in any vigorous physical activities on the day preceding the study. The last meal before the study was standardized and given at 18h00 the day before the study. The protocol was approved by the ethic committee of the IRCM and a signed consent was obtained before each study.

Experimental Design

Altogether, twenty-four (24) experiments were performed. All studies were started at 8h00 AM. The exercise consisted of 2 hours (9h00 to 11h00) of mild to moderate intensity (40% VO_2 max) using a bicycle ergometer. VO_2 max was evaluated during the week prior to the study by direct open air circuit (Q-Plex, Quinton) using a graded protocol on a bicycle ergometer (Monark) (24). Resting as well as exercise ECG was recorded at the same time to rule out cardiovascular disease.

During exercise, endogenous insulin and glucagon were suppressed by SRIF (kindly provided by Ferring Inc., Willowdale, Ontario) infused at 0.1 µg/kg•min throughout the study. Insulin (Velosulin Human R, Nordisk Gentofte Canada Inc., Mississauga, Ontario) and glucagon (Lilly Research Laboratories, Indianapolis, Indiana) were then replaced singly or in combination (insulin at 0.05 mU/kg•min and glucagon at 1.0 ng/kg•min from 0 to 60 min and at 1.5 ng/kg•min from 60 to 120 min) so as to

duplicate the levels of pancreatic hormones obtained in control experiments where saline was infused. However, since glucagon is mainly an hepatic hormone, the aim was to reproduce portal levels of the hormone which are pivotal for the action of glucagon on hepatic metabolism. On the other hand, since insulin acts mainly in the periphery, the aim of insulin replacement was to achieve peripheral levels observed in saline-treated exercise controls. Experiments were also done in resting subjects during saline infusion. Blood samples were drawn every 10 minutes throughout the last hour of exercise.

The following protocols were performed: **protocol 1**: resting controls where saline was infused throughout the 3-hour study period with the subject seated (n=3); **protocol 2:** exercise controls where saline was infused throughout the study without hormonal modification and referred to in the text as saline-treated exercise controls (n=4); **protocol 3:** exercise during insulin and glucagon deficiencies induced by SRIF infusion (n=4); **protocol 4:** exercise during glucagon deficiency induced by SRIF+insulin replacement (n=5); **protocol 5:** exercise during insulin deficiency induced by SRIF+glucagon replacement (n=5); **protocol 6:** validation of the experimental model where insulin and glucagon were both replaced during SRIF infusion (n=3).

During protocols 3 and 4 where glucagon was made deficient, subjects were hooked up to the Biostator to prevent blood glucose to fall below 3.5 mmol/L during exercise to avoid a rise in counterregulatory hormones.

A first catheter (20-gauge teflon) was placed into a forearm vein for the infusion of saline, SRIF, D_5-glucose and pancreatic hormones and a second catheter (20-gauge) was placed into a hand vein and kept at 68°C to provide arterialized venous blood for sampling (25). The cannula for sampling was kept patent with a saline drip. All reagents were prepared on the morning of each experiment. Pyrogene free D_5-glucose, SRIF, insulin and glucagon were dissolved separately in 0.9% NaCl. Hormone preparations contained approximately 1 ml of the subject's own serum to minimize hormone adhesion to tubing. All infusions were standardized using Harvard Syringe pumps. Total blood

loss for each study was less than 250 ml. In order to maintain an exercise intensity of 40% VO_2 max at a cycling frequency of 50 rpm, the load on the bike was checked every five minutes and adjusted if necessary. Blood pressure and heart rate were registered at 30 minute intervals throughout the experiment. The exercise was performed in a temperature-controlled room set at 22°C for the subject's comfort.

Tracer Methods

Glucose turnover was measured using the stable isotope D-[2,3,4,6,6-$^2H_5$]-glucose (Merck Sharp and Dome, Pointe Claire, Québec, Canada). This glucose isotope was chosen because we wanted to measure simultaneously both glucose turnover and gluconeogenesis described in a previous paper (26). The volume of distribution was calculated from a dilution technique and was found to be equal to 22% of the subject's body weight.

D-[2,3,4,6,6-$^2H_5$]-glucose was given as a prime-constant infusion with a bolus of 250 mg followed by a constant infusion at a rate of 3.0 mg/min. This dose was calculated to obtain an isotopic enrichment between 1 and 2%. The D-[2,3,4,6,6-$^2H_5$]-glucose was started at 8h00 AM, one hour before exercising and carried out through the 2-hour exercise period (9h00 to 11h00). Only data collected during the last hour of exercise was used for calculation of glucose turnover, thus allowing 2 hours for isotope equilibration.

Exact infusion rates were determined for each single experiment by measuring the isotope concentration in the infusate (D-[2,3,4,6,6-$^2H_5$]-glucose) using the mass spectrometry and multiplying by the calibrated infusion rate.

Laboratory Methods

D-d_5-glucose was separated from lactate and amino acids by column chromatography (27). The isotope was then measured by gas chromatography-mass spectrometry under electron impact in the mode of selected ion monitoring (Hewlett Packard model 5890 (GC) and 5970 (MS)). D-d_5-glucose was analyzed at its 6-acetyl-

[1,2:3,5]-bis-butaneboronyl-α-D-glucofuranose derivative (28) and ions at *m/z* 297 and 302 were measured.

After deproteinization with 6% PCA, plasma glucose was analyzed by the hexokinase method (29). Plasma FFA were measured by a calorimetric method (Wako Pure Chemical Industries, LTD). Plasma for glucagon analysis was placed in prechilled tubes containing 50 µl of aprotinin Trasylol 10 000 K.I.U./ml (Miles Canada Inc. no. 817113). Plasma insulin and glucagon were measured by radioimmunoassay (Biodata, NCS Diagnostics Inc.).

Calculations

During exercise, the isotopic steady state was disrupted and therefore the non-steady-state equations of Steele (30) were used to calculate glucose kinetics based on one compartment model of glucose kinetics. For calculations, the enrichment factor (ef) was used where ef = R / (R + 1) and $R = I_1/I_u$. I_1 represents the intensities of (M-butyl)$^+$ ion at *m/z* corresponding to labeled glucose (*m/z* = 302), and I_u the intensities of (M-butyl)$^+$ ion at *m/z* corresponding to unlabeled glucose (*m/z* = 297). Each R value was obtained by direct measurement on the mass spectrometry and corrected by standard curves before using them in the formula, thus, correcting for the isotopic purity of the tracer and the natural abundance contribution to the measured *m/z* ratios. Standard curves, obtained by weighing known amounts of labeled and unlabeled compounds were done for every new batch of tracer. The following equation for glucose turnover was used:

$$Ra = \frac{(F)}{ef} - \frac{N(def)}{ef(dt)}$$

$$Rd = Ra - \frac{(dN)}{(dt)}$$

where Ra is the rate of appearance (µmol/kg•min) and Rd the rate of utilization (µ mol/kg•min). N = P•V•G, where P is the pool fraction (0.65), V the volume of

distribution (ml), and G the blood glucose concentration (μmol/ml). F is the rate of infusion of stable isotope (μmol/min), d the delta, and t the time (minutes). The glucose metabolic clearance rate was calculated as the quotient of Rd and the arterial glucose concentration.

Statistical Analysis

Data are expressed as means ± SEM. Since all data measured were relatively stable over the second hour of exercise, we have chosen to express them as a mean over that exercise period. Therefore, for statistical purposes, the data presented here were computed for the last hour of the exercise period (from time 60 to 120 minutes). Since all subjects did not participated in all six protocols, each experiment is considered as a different subject for analytical purposes. Therefore, statistical comparisons were made using one-way analysis of variance (ANOVA) and differences between experimental groups were determined using t-test with Bonferroni correction for multiple testing using 0.05 level as statistical significance (31). However, analysis of variance (ANOVA) using repeated measures were also done for the three subjects who completed all six protocols. The level of significance was the same for both types of analysis.

RESULTS

Descriptive Data

The subject's mean BMI was 21.9 ± 0.3 kg/m^2 with a mean VO_2 max of 41.67 ± 0.85 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$. The analysis of their nutritional diaries confirmed that they followed the dietary instructions and maintained a mean caloric intake of 2474 ± 87 kCal (from 1539 to 3203) distributed as follow: 49.1 ± 0.6% carbohydrates, 32.0 ± 0.7% lipids, and 19.0 ± 0.4% proteins. None of the subjects participated in vigorous exercise on the day before the experiment.

Pancreatic Hormones (Fig. 1)

During exercise under saline infusion (protocol 2), the plasma insulin concentration decreased to 70.7 ± 1.7 pmol/L compared to 98.3 ± 10 pmol/L in the resting state. When insulin was made deficient with (protocol 3) or without glucagon deficiency (protocol 5), SRIF infusion at 0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ suppressed plasma insulin to the lower limit of the assay at 37.3 ± 1.7 and 36.3 ± 0.2 pmol/L respectively. When insulin was replaced in the absence (protocol 4) or presence of glucagon (protocol 6), insulin infused exogenously at 0.05 $mU \cdot kg^{-1} \cdot min^{-1}$ achieved plasma levels of 68.8 ± 2.3 and 73.6 ± 1.5 pmol/L, respectively and both levels were significantly lower than the resting levels (protocol 1), but similar to those observed in the exercise control (protocol 2).

During exercise under saline infusion (protocol 2), the plasma glucagon concentration increased from resting value of 107.0 ± 2.9 to 127.3 ± 4.4 ng/L but did not reach statistical significance. When glucagon was made deficient by SRIF infusion in the absence (protocol 3) or presence of insulin (protocol 4), plasma glucagon levels were decreased to the lower limit of the assay (37.1 ± 4.3 and 40.8 ± 2.6 ng/L, respectively). When glucagon was replaced at rates of 1.0 and 1.5 $ng \cdot kg^{-1} \cdot min^{-1}$ in the absence (protocol 5) or presence of insulin (protocol 6), peripheral levels of 229.9 ± 19.0 and 251.8 ± 1.0 ng/L were obtained, respectively. These levels are good approximation of the

portal levels estimated from the exercise control condition based on the portal and peripheral levels reported by Blackard et al. (32, 33).

Plasma Glucose (mmol/L) (Fig. 2)

Plasma glucose was maintained during two hours of mild to moderate ergocycle exercise (40% VO_2 max) at the levels reported in the resting protocol (4.26 ± 0.13 versus 4.46 ± 0.12 mmol/L) (Fig. 2). When glucagon was made deficient without (protocol 3) or with (protocol 4) insulin replacement, plasma glucose decreased further to 3.74 ± 0.19 and 3.85 ± 0.10 mmol/L respectively. In these two protocols, however, plasma glucose was prevented from decreasing to lower levels by the Biostator. In protocols 3 and 4, the Biostator infused a mean of 6.88 ± 1.15 and 7.52 ± 1.19 $\mu \cdot kg^{-1} \cdot min^{-1}$ of dextrose, respectively, during the last hour of exercise. This suggests that glucagon plays a crucial role in maintaining plasma glucose during mild to moderate exercise. When insulin was made deficient in the presence of portal levels of glucagon (protocol 5), plasma glucose increased significantly to 6.69 ± 0.68 mmol/L, suggesting that insulin plays an important role in preventing hyperglycemia during exercise.

Glucose Uptake (Figs. 3 and 4, Table I)

In all protocols, glucose utilization increased during exercise. When both pancreatic hormones were deficient during exercise (protocol 3), *Rd* was lower (18.6 ± 3.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) but remained 1.5 times higher than the resting level (12.1 ± 0.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). However, when glucagon was made deficient in the presence of insulin (protocol 4), *Rd* was similar to the level measured in the saline-treated exercise controls (25.9 ± 1.5 versus 28.6 ± 3.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). When glucagon was infused with insulin deficiency (protocol 5), *Rd* reached its highest level at 36.6 ± 4.9 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Finally, the levels obtained during replacement of both insulin and glucagon (protocol 6) were similar (32.1 ± 2.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) to those measured in the saline-treated exercise controls.

In the Figure 4 and bottom of Table I are shown the glucose metabolic clearance rates where glucose uptake is corrected for glucose concentration. The data indicate that even when insulin was made deficient in protocols 3 and 5 glucose clearance was increased 2-fold over resting values (4.8 ± 0.3 and 5.4 ± 0.4 versus 2.7 ± 0.7 $ml \cdot kg^{-1} \cdot min^{-1}$). When insulin was present, however, even at lower concentrations during exercise (protocols 2, 4 and 6), glucose clearance was increased 3-fold at 6.9 ± 0.9, 6.9 ± 0.3 and 7.7 ± 0.6 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively.

Plasma FFA (Table II)

Plasma free fatty acids increased during exercise in all protocols. In the saline-treated exercise controls (protocol 2) and when insulin and glucagon were both replaced during SRIF infusion (protocol 6), the increase in FFA did not reach significance (0.601 ± 0.154 and 0.509 ± 0.177 mmol/L, respectively). When insulin and/or glucagon were made deficient (protocols 3, 4 and 5), however, the increase in plasma FFA were higher at 0.985 ± 0.033, 0.841 ± 0.081 and 0.895 ± 0.036 mmol/L, respectively.

DISCUSSION

This study was designed to further explore the respective roles of insulin and muscle contractions in the regulation of glucose utilization during mild to moderate intensity exercise in postabsorptive healthy male subjects. To do so, we used somatostatin to suppress the endogenous secretion of the pancreatic hormones and these were replaced singly or in combination. Our results show that muscle contraction *per se* induces glucose uptake by the exercising muscle in the absence of insulin. Insulin is important, however, to achieve maximal glucose uptake during exercise.

In the saline-treated subjects (protocol 2) mild to moderate exercise resulted in a decrease in plasma insulin and an increase in plasma glucagon (Fig. 1). The magnitude of these changes in pancreatic hormones during exercise were similar to those reported by some investigators (3, 21, 34-36), but different from those reported by others (37-39). It is possible that in the latter studies differences in the intensity and/or the duration of exercise, the level of physical fitness, the antecedent diet or the nutritional state could account for the discrepancies (17). Under our experimental conditions, mild to moderate intensity exercise for two hours was sufficient to induce a 26% decrease in insulin and a 20% increase in glucagon, changes that are consistent with other studies done under similar conditions (3, 21, 34-36).

During somatostatin infusion, glucagon and insulin levels decreased to below levels detectable by the assay, reflecting an adequate inhibition of the pancreatic hormones by somatostatin (40-42). Validation of our experimental model was dependent on the possibility of duplicating the hormonal concentrations observed in the saline-treated exercise controls and, more importantly, of reproducing the metabolic changes induced by exercise in the control group. The glucagon levels measured in protocol 5 and 6 were twice as high as those observed in the saline-treated exercise control (protocol 2) thus achieving levels similar to those observed in the portal veins (43, 44). On the other hand, the insulin levels achieved in protocol 4 and 6 were similar to those obtained

peripherally (protocol 2). But more importantly, in protocol 6, we were able to reproduce very closely the glucose utilization (32.1 ± 2.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and glucose clearance (7.7 ± 0.6 $ml \cdot kg^{-1} \cdot min^{-1}$) observed in the saline-treated exercise control group (28.6 ± 3.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and 6.9 ± 0.9 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively). Thus, we feel confident that our experimental model was appropriate to study the effects of insulin versus muscle contractions on glucose uptake.

In our study, mild to moderate exercise in healthy male subjects was associated with a 136% increase in glucose utilization and a 156% increase in glucose clearance (Figs. 3 and 4, Table I). These are in good agreement with other studies done in dogs (1, 5, 12, 14, 45, 46) and in man (2, 16, 21, 34, 35, 37). These observations support an important role for glucose as an energy-substrate for the contracting muscle during mild to moderate intensity exercise.

Interestingly, this increase in glucose uptake by the exercising muscles occurred despite a significant decrease in circulating insulin levels (Fig. 1). Therefore, the increase in glucose uptake is not due to an increase in insulin concentration. This indicate that under these conditions, the linear relationship between insulin concentration and glucose uptake is lost. Furthermore, it suggests that factors other than insulin could be implicated in the increase glucose uptake during exercise.

This is further supported by our observation that glucose uptake is still increase during exercise when insulin is made deficient (protocols 3 and 5). This observation indicates that in the absence of insulin, muscle contraction *per se* can stimulate glucose uptake during exercise. This would be compatible with the data from Sternlicht et al. (47) who showed that muscle contraction *per se* can induce the translocation of GLUT4 from muscle sarcoplasm to the plasma membrane. Our data are therefore compatible with other data from the literature suggesting that muscle contraction and insulin can stimulate glucose transport in skeletal muscle by separate pathways (47-51). *In vitro*, glucose transporter can be translocated by the muscle contraction or insulin (52-54).

Furthermore, results from isolated muscle suggest that muscle contractions *per se* are sufficient to increase glucose uptake (8-11). In these studies, however, no direct measurement of sugar transporters were performed. Because it has been shown that a small increase in cytosolic Ca^{2+} concentration was able to increase glucose transport in skeletal muscle, it could be hypothesized that increase in cytosolic Ca^{2+} concentration associated with muscular contraction is sufficient to increase glucose transport (56).

In our study, mild to moderate intensity exercise was associated with an increase in plasma free fatty acids (Table II). These results are in agreement with those reported by other investigators (21, 55). The highest levels of plasma FFA were achieved in the absence of insulin (protocols 3 and 5) confirming that the reduced insulin availability is an important determinant of lipolysis during exercise (19). From our results, it is impossible to determine whether the increased lipolytic activity associated with insulin deficiency is responsible for the lower glucose uptake value observed in protocol 3, or whether the lower glucose uptake level leads to a compensatory increase in FFA utilization. It has been shown that the exercise-induced rise in catecholamines are needed for maximum activation of lipolysis (19, 46). In our study, it is likely the β-adrenergic activity is the predominant factor stimulating lipolysis. This most likely explain the small and non significant difference in FFA concentrations between protocol without and with insulin in the presence of SRIF. It is possible that the increase plasma FFA exert a restraining effect on glucose uptake by the exercising muscle. This would be in line with Hargreaves et al. (56) observation that muscle glucose uptake is reduced by increasing FFA. However, we could not show a linear negative correlation between glucose utilization and FFA levels.

From our study, it is concluded that muscle contraction *per se* is a major determinant of glucose uptake by the exercising muscle. It would appear, however that insulin is important to achieved maximal glucose uptake during prolonged mild to moderate exercise in postabsorptive healthy subjects. It is therefore suggested that during

mild to moderate intensity exercise, a good proportion of the glucose is taken up by the exercising muscle independently of circulating insulin.

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TABLE I

GLUCOSE UTILIZATION AND METABOLIC GLUCOSE CLEARANCE RATE

PROTOCOLS	REST	EXERCISE				
	1 Saline	2 Saline	3 SRIF	4 SRIF+INS	5 SRIF+GLUC	6 SRIF+ INS+GLUC
		GLUCOSE UTILIZATION ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)				
Subjects						
1	11.5	32.4	27.3	27.6	26.1	29.8
2		34.6	17.4	17.4	29.9	
3	12.9	21.1	16.4	26.4	48.9	29.9
4	11.9	26.3	13.3	20.9	48.2	36.6
5				24.5	30.2	
Mean ± SEM	12.13±0.43[bdef]	28.6±3.1[ac]	18.6±3.0[bef]	25.9±1.5[ae]	36.7±4.9[acd]	32.1±2.2[ac]
		GLUCOSE METABOLIC CLEARANCE RATE ($ml \cdot kg^{-1} \cdot min^{-1}$)				
Subjects						
1	2.6	8.5	6.9	7.3	5.3	6.5
2		8.1	5.4	7.3	5.3	
3	2.8	4.7	4.2	7.4	5.7	8.4
4	2.6	6.3	2.7	5.9	6.7	8.1
5				6.4	3.9	
Mean ± SEM	2.7±0.7[bcdef]	6.9±0.9[a]	4.8±0.9[ab]	6.9±0.3[ac]	5.4±0.4[a]	7.7±0.6[ace]

[a]Significantly different at $p < 0.05$ from resting control
[b]Significantly different at $p < 0.05$ from exercise control
[c]Significantly different at $p < 0.05$ from SRIF
[d]Significantly different at $p < 0.05$ from SRIF+INS
[e]Significantly different at $p < 0.05$ from SRIF+GLUC
[f]Significantly different at $p < 0.05$ from SRIF+INS+GLUC

TABLE II

PLASMA FREE FATTY ACIDS

PROTOCOLS	REST	EXERCISE				
	1 Saline	2 Saline	3 SRIF	4 SRIF+INS	5 SRIF+GLUC	6 SRIF+ INS+GLUC
			PLASMA FREE FATTY ACIDS (mmol/L)			
Subjects						
1	0.202	0.404	0.972	0.635	0.891	0.567
2		0.601	0.985	0.731	0.940	
3	0.389	0.361	0.912	1.114	0.982	0.676
4	0.325	1.037	1.071	0.836	0.895	0.283
5				0.887	0.767	
Mean ± SEM	0.305±0.055[cde]	0.601±0.154[c]	0.985±0.033[abe]	0.841±0.081[af]	0.895±0.036[af]	0.509±0.117[cde]

[a]Significantly different at $p < 0.05$ from resting control
[b]Significantly different at $p < 0.05$ from exercise control
[c]Significantly different at $p < 0.05$ from SRIF
[d]Significantly different at $p < 0.05$ from SRIF+INS
[e]Significantly different at $p < 0.05$ from SRIF+GLUC
[f]Significantly different at $p < 0.05$ from SRIF+INS+GLUC

FIGURES LEGEND

Figure 1

The effects of exercise at 40% VO_2 max on plasma glucose during the infusion of saline (protocol 2), SRIF (insulin and glucagon deficiencies - protocol 3), SRIF + insulin (glucagon deficiency - protocol 4), SRIF + glucagon (insulin deficiency - protocol 5), and SRIF + exogenous replacement of insulin and glucagon (protocol 6) compared to the resting state (protocol 1). The data are expressed as means ± SEM. a: Significantly different at $p < 0.05$ from resting control; b: significantly different at $p < 0.05$ from exercise control; c: significantly different at $p < 0.05$ from SRIF; d: significantly different at $p < 0.05$ from SRIF + INS; e: significantly different at $p < 0.05$ from SRIF + GLUC; f: significantly different at $p < 0.05$ from SRIF + INS + GLUC.

Figure 2

The effects of exercise at 40% VO_2 max on plasma glucose during the infusion of saline (protocol 2), SRIF (insulin and glucagon deficiencies - protocol 3), SRIF + insulin (glucagon deficiency - protocol 4), SRIF + glucagon (insulin deficiency - protocol 5), and SRIF + exogenous replacement of insulin and glucagon (protocol 6) compared to the resting state (protocol 1). The data are expressed as means ± SEM. a: Significantly different at $p < 0.05$ from resting control; b: significantly different at $p < 0.05$ from exercise control; c: significantly different at $p < 0.05$ from SRIF; d: significantly different at $p < 0.05$ from SRIF + INS; e: significantly different at $p < 0.05$ from SRIF + GLUC; f: significantly different at $p < 0.05$ from SRIF + INS + GLUC.

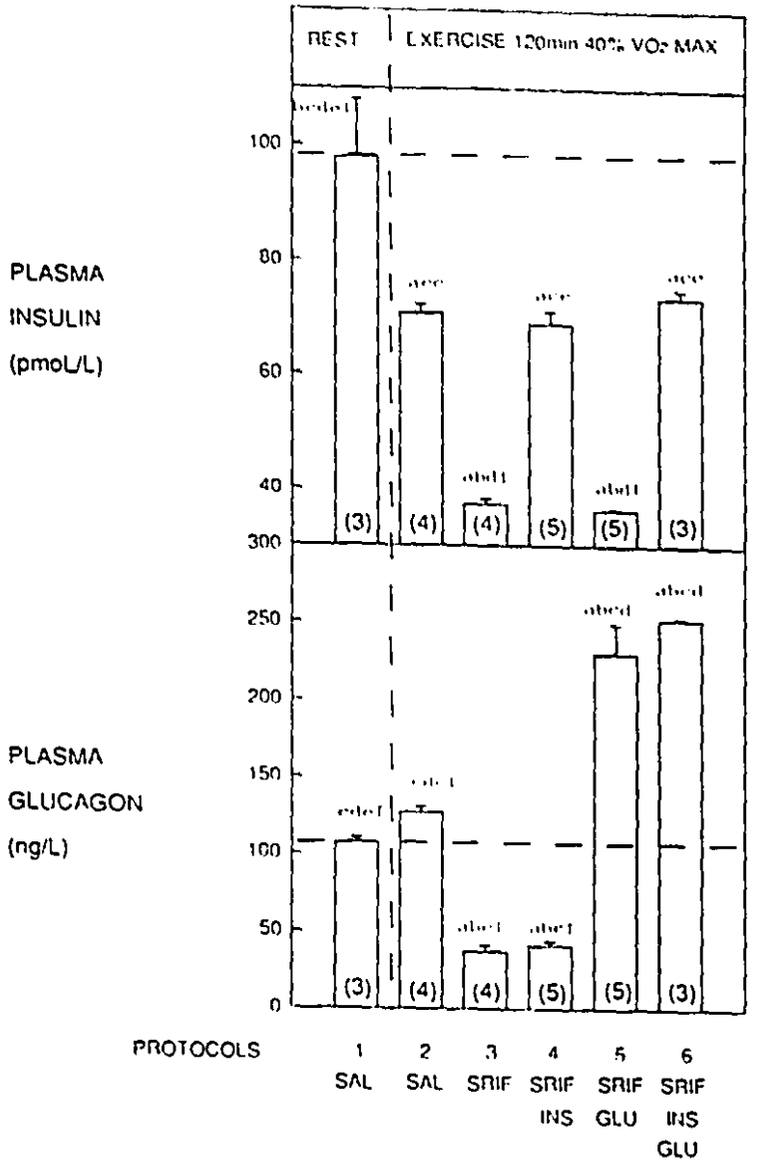
Figure 3

The effects of exercise at 40% VO_2 max on glucose utilization during the infusion of saline (protocol 2), SRIF (insulin and glucagon deficiencies - protocol 3), SRIF + insulin (glucagon deficiency - protocol 4), SRIF + glucagon (insulin deficiency - protocol 5), and SRIF + exogenous replacement of insulin and glucagon (protocol 6) compared to

the resting state (protocol 1). The data are expressed as means ± SEM. a: Significantly different at $p < 0.05$ from resting control; b: significantly different at $p < 0.05$ from exercise control; c: significantly different at $p < 0.05$ from SRIF; d: significantly different at $p < 0.05$ from SRIF + INS; e: significantly different at $p < 0.05$ from SRIF + GLUC; f: significantly different at $p < 0.05$ from SRIF + INS + GLUC.

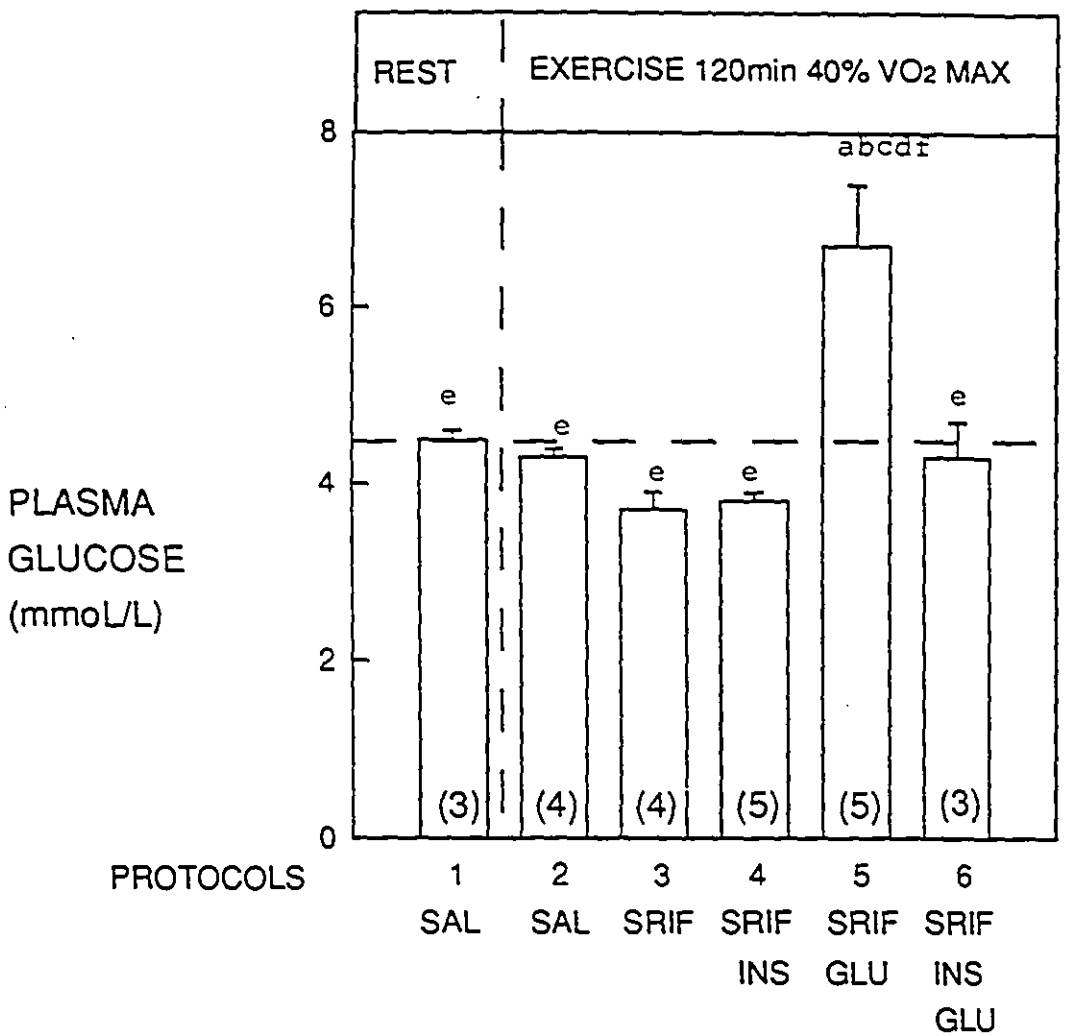
Figure 4

The effects of exercise at 40% VO_2 max on glucose metabolic clearance rate during the infusion of saline (protocol 2), SRIF (insulin and glucagon deficiencies - protocol 3), SRIF + insulin (glucagon deficiency - protocol 4), SRIF + glucagon (insulin deficiency - protocol 5), and SRIF + exogenous replacement of insulin and glucagon (protocol 6) compared to the resting state (protocol 1). The data are expressed as means ± SEM. a: Significantly different at $p < 0.05$ from resting control; b: significantly different at $p < 0.05$ from exercise control; c: significantly different at $p < 0.05$ from SRIF; d: significantly different at $p < 0.05$ from SRIF + INS; e: significantly different at $p < 0.05$ from SRIF + GLUC; f: significantly different at $p < 0.05$ from SRIF + INS + GLUC.



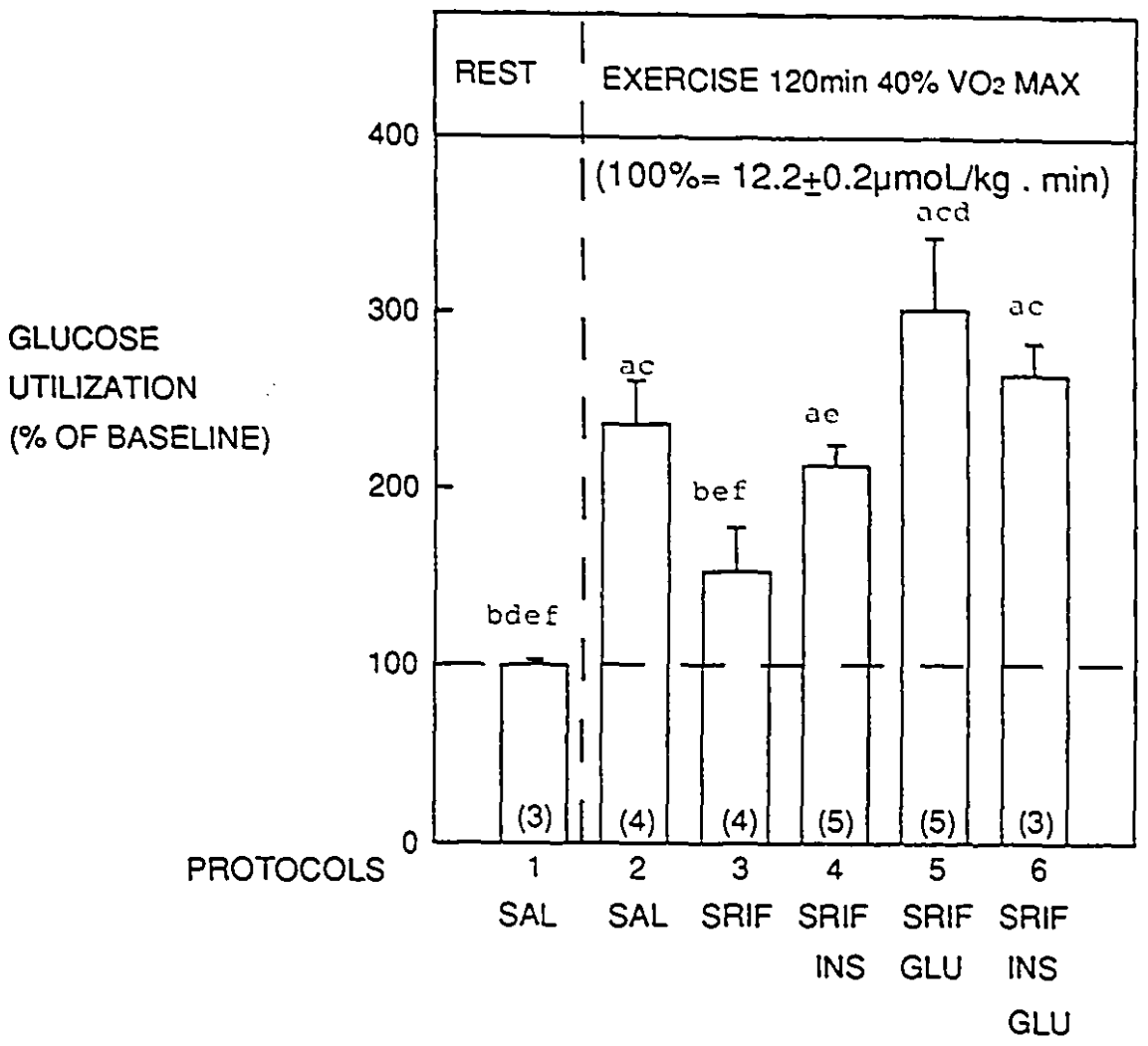
[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

FIGURE 1



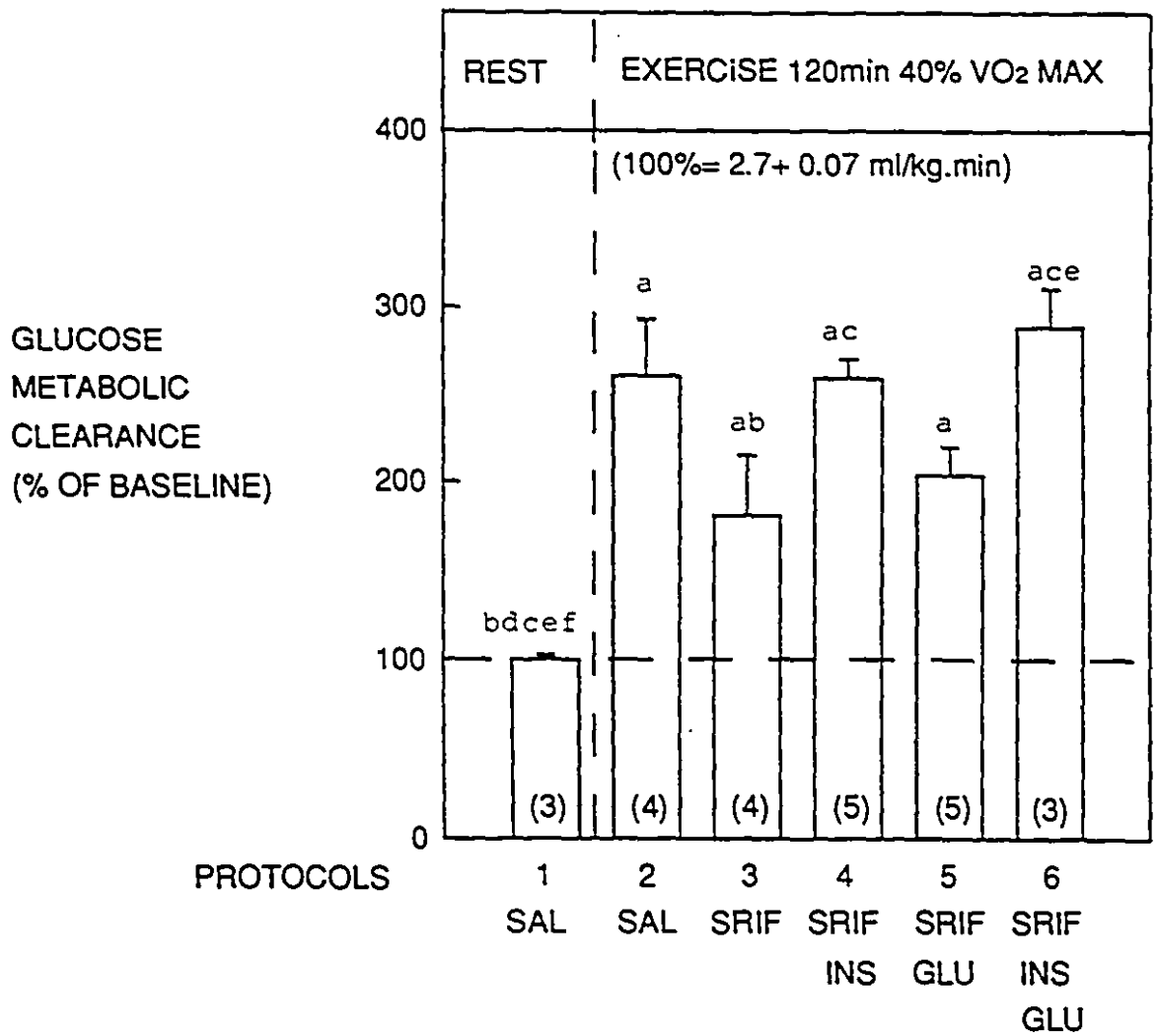
[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

FIGURE 2



[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

FIGURE 3



[a] Significantly different at $p < 0.05$ from resting control
[b] Significantly different at $p < 0.05$ from exercise control
[c] Significantly different at $p < 0.05$ from SRIF
[d] Significantly different at $p < 0.05$ from SRIF + INS
[e] Significantly different at $p < 0.05$ from SRIF + GLUC
[f] Significantly different at $p < 0.05$ from SRIF + INS + GLUC

FIGURE 4

CHAPTER 5

DISCUSSION

This study was designed to further explore and dissect the role of insulin and glucagon in the regulation of glucose fluxes during mild to -moderate intensity exercise in healthy male subjects. To do so, we used SRIF to suppress the endogenous secretion of insulin and glucagon, and the pancreatic hormones were replaced singly (SRIF+INS or SRIF+GLUC) or in combination (SRIF+INS+GLUC) to match the hormonal concentrations observed during exercise in control subjects. The roles of insulin and glucagon in the regulation of glucose fluxes during mild to moderate exercise were evaluated using a double stable isotope technique validated in our laboratory to measure: 1) hepatic glucose production; 2) gluconeogenesis; 3) glucose utilization; and 4) lipolysis; during a 120 minutes ergocycle exercise at 40% VO_2 in a postabsorptive state.

We presented three manuscripts which represent the totality of the data and are being prepared to be submitted for publication. We decided to separate them as following: the first one deals with the regulation of hepatic glucose production, the second with the regulation of gluconeogenesis, and the last one with the regulation of glucose utilization and lipolysis.

Prior to this study, we were conscious of the fact that the isotopic techniques utilized for calculating gluconeogenesis, from the conversion of any precursor into glucose, bring in minimal appraisal due to isotopic dilution in the oxaloacetate pool. The dilution in the oxaloacetate pool comes from either the exchange of the labeled carbons of the chosen glucose precursors within the hepatocyte by unlabeled carbons originating from acetyl coenzyme A and/or the loss as CO_2 (Consoli and Nurjhan, 1990). For this reason, we have presented our results in terms relative to the basal situation. It is important to note that the technique of concomitant utilization of D-[2,3,4,6,6-$^2H_5$]-glucose also yields a theoretical overestimation of glucose production and utilization resulting from the loss of deuterium from position 2 in the first futile cycle (Wolfe, 1988). For this reason, this technique gives an overvaluation of gluconeogenesis. However, this

factor is well counterbalanced by the OAA dilution mentioned previously providing an under evaluation of the true gluconeogenic rate (Hetenyi, 1986). This method provides us only with an index of gluconeogenesis and not a true quantification of this metabolic process.

Our exercise protocol of mild to moderate intensity exercise for two hours was sufficient to induce a 26% decrease in insulin and a 20% increase in glucagon, hormonal changes that are quite consistent with other studies done under similar conditions (Felig and Wahren, 1979; Wolfe et al., 1986; Shilo et al., 1990; Hirsch et al., 1991;Wasserman et al., 1991).

In the presence of SRIF, glucagon and insulin attained their lowest levels detectable by the assay, reflecting an adequate inhibition by somatostatin as shown previously (Cherrington et al., 1977, 1983; Stevenson et al., 1984). We did not replace growth hormone during this study. It is well known that the hyperglycemic action of growth hormone is delayed for several hours (MacGorman et al., 1981); therefore, it is most unlikely that growth hormone would play an important glucoregulatory role in the acute exercise response such as the one investigated in the present study. Blood samples for catecholamines were included initially in this study but, unfortunately, these samples were lost. By the time we realized this inconvenience it was to late to modify the protocol.

Validation of our experimental model was dependent on the possibility of duplicating the hormonal concentrations observed in the saline-treated exercise controls and, more importantly, of reproducing the metabolic changes induced by exercise. In protocol 6 (SRIF+INS+GLUC), we were able to reproduce the levels of pancreatic hormones observed in protocol 2 (saline-treated exercise control), as well as metabolite concentrations (lactate, alanine, β-hydroxybutyrate, glycerol and free fatty acids) and

glucose utilization, metabolic clearance rate, hepatic glucose production and gluconeogenesis values. Thus, we feel confident that our experimental model was appropriate to study the effects of pancreatic hormones replacement on glucose fluxes.

It is important to remember that portal glucagon levels are twice as high as peripheral levels and that portal glucagon levels are pivotal for the action of the hormone on hepatic metabolism. Thus, the aim of glucagon replacement was to reproduce portal levels peripherally. Because insulin acts mainly in the periphery, the aim of insulin replacement was to duplicate peripheral levels. Experiments were also done in resting subjects during saline infusion. It is crucial to remember that although our glucagon concentrations, measured during protocol 5 (SRIF+GLUC: 222.9 ± 19 ng/L) and 6 (SRIF+INS+GLUC: 251.8 ± 1.0 ng/L), were twice as high as those observed in protocol 2 (saline-treated exercise control: 127.3 ± 4.4 ng/L), this difference was intentional. Portal glucagon levels are twice as high as peripheral levels and it is the portal levels that are pivotal for the action of the hormone on hepatic metabolism. Thus, the aim of glucagon replacement was to reproduce portal levels peripherally which was attained in the present study. When calculating the portal to peripheral (protocol 5 and 6) gradients we achieved a value similar to the ones previously reported by others (Felig et al., 1974; Greco et al., 1979). Because insulin acts mainly in the periphery, the aim of insulin replacement was to duplicate peripheral levels. In protocol 6, where both pancreatic hormones were replaced, insulin infusion resulted in peripheral insulin levels (73.6 ± 1.5 pmol/L) that were close to those observed in the exercise control condition (70.7 ± 1.7 pmol/L). These similar peripheral levels were achieved, however, at the expense of lower portal vein insulin. We are therefore quite pleased that we have reproduced the glucagon portal vein hormonal milieu observed during mild to moderate intensity exercise. In contrast to Hirsch et al. (1991), our simulated exercise-induced modification of pancreatic hormone

concentrations mimicked precisely the biologically response observed in the exercise control condition.

Furthermore, the increase in glucose utilization and glucose metabolic clearance rate reported in the third manuscript, for protocol 6, were nearly triple the resting state (32.11 ± 2.2 versus 12.13 ± 0.43 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and 7.67 ± 0.59 versus 2.65 ± 0.7 $ml \cdot kg^{-1} \cdot min^{-1}$) and comparable to the saline-treated exercise control (28.59 ± 3.06 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and 6.9 $ml \cdot kg^{-1} \cdot min$)). The same was observed for glucose production and gluconeogenesis index in the first and second manuscripts. In protocol 6, the increase in glucose production and gluconeogenesis were twice the resting state (29.3 ± 0.85 versus 12.07 ± 0.43 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and 1.049 ± 0.033 versus 0.663 ± 0.04 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and comparable to the saline-treated exercise control (27.56 ± 3.03 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ and 0.959 ± 0.116 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Once again we therefore feel quite confident that the pancreatic hormones replacement during somatostatin infusion was adequate.

Since the pioneering reports appeared in the literature, there has been a refinement of the technique used to study hormonal regulation. We contribute to original knowledge by utilizing for the first time during mild to moderate exercise a novel technique validated in our laboratory (Martineau et al., 1985). Using two stable isotopes (D-[2,3,4,6,6-$^{2}H_5$]-glucose and L-[1,2,3-$^{13}C_3$]-alanine), we measured simultaneously glucose turnover and gluconeogenesis during 2 hours of bicycle exercise at 40% VO_2 max to characterize the respective roles of insulin and glucagon in the control of these two metabolic processes. We have used SRIF to suppress the endogenous secretion of insulin and glucagon. The pancreatic hormones were either not replaced or replaced singly or in combination to match the hormonal concentrations observed during exercise in control subjects. From the available studies on hormonal regulation during exercise, it is the first time that hormonal regulation dissection during exercise was accomplished this way in healthy human subjects.

Based on our observations, it becomes obvious that it is the interaction between the two pancreatic hormones that is responsible for the fine regulation of hepatic glucose production and glucose homeostasis during exercise. We have shown in the absence of glucagon with or without the presence of insulin that the increase in hepatic glucose production induced by exercise was totally abolished. Insulin on the other hand is important in preventing an overshoot in the exercise-induced increase in hepatic glucose production which could ultimately result in hyperglycemia. From our data, we estimate that glucagon would be responsible for 40% of hepatic glucose production during exercise while insulin would account for a 22% suppression of the glucagon stimulated increase in hepatic glucose production (manuscript 1).

From the experiment described (manuscript 1), it is concluded that glucagon plays a preponderant role in the exercise-induced increase in hepatic glucose production. However, lower insulin levels observed during exercise are important in preventing an overproduction of glucose by the liver and the subsequent development of hyperglycemia. It is therefore the interaction between the two pancreatic hormones that precisely regulates the increase in hepatic glucose production during exercise.

Prior to this study, very little was known about hormonal regulation of gluconeogenesis in healthy subjects during exercise. We were able to infuse both pancreatic hormones to assess the specific roles of the exercise-induced rise in glucagon and fall in insulin levels. Our results (manuscript 2) demonstrate for the first time in postabsorptive healthy subjects that glucagon is crucial for the stimulation of gluconeogenesis during mild to moderate intensity exercise. Our data are the first to describe the hormonal regulation of hepatic gluconeogenesis during exercise in healthy postabsorptive male subjects. Although *in vivo* studies can not quantitate the effects of glucagon and insulin on gluconeogenesis, our study shows clearly that the exercise-induced increase in the level of glucagon significantly stimulates that process and that the

presence of glucagon is crucial for a gluconeogenic response to exercise. We can also suggested that the lower level of insulin during exercise still exert a restraining effect on glucagon stimulated gluconeogenesis.

Although there is little doubt that muscle contractions *per se* can enhance glucose uptake *in vitro* (Nesher et al., 1985; Ploug et al., 1987, 1992), the precise role of the interaction between muscle contractions and insulin *in vivo* remains unclear especially in humans. In the present study, we studied in healthy subjects the role of insulin in the regulation of glucose utilization during exercise. In all exercise conditions, a more than 1.5-fold increase in glucose utilization was noted and our values were similar to the ones reported by other groups (Wolfe et al., 1986; Shilo et al., 1990; Hirsch et al., 1991). Wasserman and colleagues (1991) showed that virtually the entire exercise-induced increase in *Rd* occurred independently of acute insulin action. Although, it did not reach significance, exercise without insulin and glucagon replacement (protocol 3) induced a 53% increase in glucose utilization. Exercise doubled glucose metabolic clearance rate in the absence of insulin; results which are of the same order of magnitude as those reported by Björkman et al. (1988). From our results, we estimated that about half of metabolic clearance rate of glucose was insulin independent, indicating that with insulin deficiency *in vivo* muscle contractions can stimulate glucose uptake. It is possible that some process inherent to muscle contractions is responsible for the increase in glucose utilization. However, as noted by other groups (Zorzano et al., 1986; Constable et al., 1988; Wallberg-Henriksson et al., 1988), insulin appears to be necessary to duplicate exactly the exercise control response in glucose uptake. From our results, we conclude that muscle contraction *per se* is a major determinant of glucose uptake by the exercising muscle. It would appear, however, that insulin is important to achieve maximal complete glucose uptake during prolonged mild to moderate exercise in postabsorptive healthy subjects. It is therefore suggested that during mild to moderate intensity exercise, a good proportion

of the glucose is taken up by the exercising muscles independently of the circulating insulin.

From the observations of our manuscripts, we could answer our initial hypotheses that the exercise-induced rise in hepatic glucose production is dependent on the exercise-induced increase in glucagon and in part on the decrease in circulating insulin. The exercise-induced increase in glucose utilization is not totally dependent on circulating insulin; the presence of insulin however being required for the maximal expression of glucose uptake. However, we were not able to show any significant inverse correlation between glucose uptake and circulating levels of FFA.

In conclusion, although the techniques used bear some limitations, the present study provides good evidence that the role of glucagon is clearly preponderant for the stimulation of glucose production while insulin is important but less crucial for the increase in hepatic glucose production and gluconeogenesis during exercise at 40% VO_2 max in healthy subjects. We have also shown that the decreased insulin levels during exercise play an important role in restraining the rise in hepatic glucose production induced by exercise. Moreover, we have shown that insulin was not essential for glucose uptake during mild to moderate intensity exercise in postabsorptive healthy subjects. This further emphasizes that muscle contractions *per se* can stimulate glucose uptake. Insulin is important, however, for maximal glucose uptake by the contracting muscle. To confirm our conclusions, further research on the effects of exercise and insulin stimulation on skeletal muscle glucose transporters *in vivo* will be required. Future progress in this area will depend upon molecular identification of the interaction of insulin and exercise which will provide the tools to understand the mechanisms involved in the regulation of glucose metabolism.

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APPENDIXES

I. PROTOCOL

Overnight fasted (approximately 15-hour postabsorptive), healthy male subjects within 10% of their ideal body weight participated in a 2-hour exercise of mild to moderate intensity. All subjects were instructed to follow a well-balanced diet (50% carbohydrates, 35% lipids, and 15% proteins) and to maintain a nutritional diary including daily physical activities during the week prior to the study. They were also instructed not to participate in any vigorous physical activities on the day preceding the study. Their nutritional diary was analyzed using Miles Canada Inc. program. The last meal before the study was standardized and given at 18h00 the day before the study.

Every subject had a normal 2-hour OGTT, a normal history and physical exam. They also had normal ECG and chest X-ray. The complete blood count, biochemistry profile and liver function test were normal. The protocol was approved by the ethic committee of the IRCM and informed consent was obtained before each study.

A. Experimental Design

Altogether, twenty-four (24) experiments were performed. All studies were started at 8h00 AM. The protocol consisted of 2 hours (9h00 to 11h00) of mild to moderate intensity (40% VO_2 max) exercise using a bicycle ergometer. VO_2 max was evaluated during the week before the study with a direct open air circuit (Quinton Instrument Company, Q-Plex Cardio-pulmonary exercise system) using a graded protocol (ACSM, 1991) on a bicycle ergometer (Monark, Sweden). Every 2 minutes, the exercise intensity was increased by 150 kpm/min. Criteria for achievement of VO_2 max were the ones described by the ACSM. Among them, the failure to keep up with the frequency of pedaling (50 rpm) and/or the leveling off of oxygen consumption despite increasing intensity were the most frequent (ACSM 1991). Resting as well as exercise ECG

(Quinton Instrument Company, Stress test monitors Quinton Q5000) were recorded at the same time to rule out cardiovascular disease.

During exercise, endogenous insulin and glucagon were suppressed by SRIF (kindly provided by Ferring Inc. Willowdale, Ontario) at an infusion rate of 0.1 μg/kg•min throughout the study. Insulin (Velosulin Human R, Nordisk Gentofte Canada Inc., Mississauga, Ontario) and glucagon (Lilly Research Laboratories, Indianapolis, IN) were then replaced singly or in combination (insulin at 0.05 mU/kg•min and glucagon: from 0 to 60 min at 1.0 ng/kg•min and from 60 to 120 min at 1.5 ng/kg•min) so as to duplicate the levels of pancreatic hormones obtained in control experiments where saline was infused during exercise. However, since glucagon is mainly an hepatic hormone, it is important to remember that portal glucagon levels are twice as high as peripheral levels and that portal glucagon levels are pivotal for the action of the hormone on hepatic metabolism. Thus, the aim of glucagon replacement was to reproduce portal levels peripherally. On the other hand, since insulin acts mainly in the periphery, the aim of insulin replacement was to achieve peripheral levels observed in saline-treated exercise controls. Experiments were also done in resting subjects during saline infusion.

The following protocols were performed:

Protocol 1 (saline-treated resting control)

Resting control where saline was infused throughout the 3-hour study period with subjects seated.

Protocol 2 (saline-treated exercise control)

Exercise control where saline was infused throughout the study without hormonal modification.

Protocol 3 (SRIF exercise)

Exercise with insulin and glucagon deficiencies induced by SRIF infusion.

Protocol 4 (SRIF+insulin exercise)

Exercise with glucagon deficiency induced by SRIF + insulin replacement (peripheral level).

Protocol 5 (SRIF+glucagon exercise)

Exercise with insulin deficiency induced by SRIF + glucagon replacement (portal level).

Protocol 6 (SRIF+insulin+glucagon exercise)

Validation of the experimental model where insulin and glucagon were both replaced during SRIF infusion.

During protocols 3 and 4 where glucagon was made deficient, subjects were hooked up to the biostator to prevent blood glucose to fall below 3.5 mmol/L during exercise to avoid the interference of counterregulatory hormones .

After subjects had voided, a first catheter (20-gauge teflon) was placed into a forearm vein for the infusion of saline, SRIF, D_5-glucose, ^{13}C-alanine and pancreatic hormones and a second catheter (20-gauge) was placed into a hand vein and kept at 68°C to provide arterialized venous blood for sampling (Abumrad et al., 1981). The cannula for sampling was retained patent with a saline drip. All reagents were prepared on the morning of each experiment. A blood sample was then drawn, before any isotope infusion, in order to determine the naturally occurring background enrichment of glucose. Pyrogene free D-[2,3,4,6,6-$^2H_5$]-glucose, L-[1,2,3-^{13}C]-alanine, SRIF, insulin and glucagon were dissolved separately in 0.9% NaCl. Hormone preparations contained approximately 1 ml of the subject's own serum to minimize hormone adhesion to the tubing. All infusions were standardized using Harvard Syringe Pumps (Harvard Apparatus, Ealing Scientific Limited, Québec, Canada). Total blood loss for each study was less than 250 ml and six (6) weeks were spanned between two consecutive studies. In order to maintain an exercise intensity of 40% VO_2 max at a cycling frequency of 50

rpm, the load on the bike was checked every five minutes and ajusted if necessary. Blood pressure, heart rate and Borg perceived exertion rate were registered at 30 minutes intervals throughout the experiment. The exercise was performed in a temperature-controlled room: temperature was set ~22°C for the subject's comfort.

II. METHODOLOGY

A. Glucose Turnover

Glucose turnover was measured using the isotope D-[2,3,4,6,6-$^2H_5$]-glucose (Merck Sharp and Dome, Pointe Claire, Québec, Canada). This glucose isotope was chosen because we wanted to measure simultaneously both glucose turnover and gluconeogenesis as described in a previous paper (Martineau et al., 1985). The volume of distribution was calculated from a dilution technique using tritiated glucose [3-^{3}H glucose] after the first hour of cycling to verify for any change in such volume during our exercise protocol and, it was found to be equal to 22% of the subject's body weight.

The D-[2,3,4,6,6-$^2H_5$]-glucose was given as a primed-constant infusion with a bolus of 250 mg followed by a constant infusion at a rate of 3.0 mg/min. This dose was calculated to obtain an isotopic enrichment between 1 and 2%. The D-[2,3,4,6,6-$^2H_5$]-glucose infusion was started at 8h00 AM, one hour before exercise and carried through the 2-hour exercise period (9h00 to 11h00). Only data collected during the last hour (from 10h00 to 11h00) of exercise was used for the calculation of glucose turnover, thus allowing 2 hours for isotopic equilibration.

Exact infusion rates were determined for each experiment by measuring the isotope concentration in the infusate (D-[2,3,4,6,6-$^2H_5$]-glucose) using the mass spectrometry (Hewlett Packard model 5890 (GC) and 5970 (MS)) and by multiplying by the calibrated infusion rate.

During exercise, the isotopic steady state is disrupted and therefore the non-steady-state equations of Steele (1959) were used to calculate glucose kinetics. Glucose appearance (*Ra*) and utilization (*Rd*) were calculated according to the method of Wall et al. (1957), as modified by De Bodo et al. (1963), Cowan and Hetenyi (1971) and Cherrington and Vranic (1973), using the unsteady state equation based on one compartment model of glucose kinetics. For calculations, the enrichment factor (*ef*) was used.

$$ef = R/(R+1) \text{ and } R = I_1/I_u$$

I_1 represents the intensities of (M-butyl)$^+$ ion at *m/z* corresponding to labeled glucose (*m/z* = 302), and I_u the intensities of (M-butyl)$^+$ ion at *m/z* corresponding to unlabeled glucose (*m/z* = 297).

Each *R* value was obtained by direct measurement on the mass spectrometry and corrected by standard curves before using them in the formula, therefore, correcting for the isotopic purity of the tracer and the natural abundance contribution to the measured *m/z* ratios. Standard curves, obtained by weighing known amounts of labeled and unlabeled compounds were accomplished for every new batch of tracer. The following equation for glucose turnover was used:

$$Ra = \frac{(F)}{ef} - \frac{N(def)}{ef(dt)}$$

$$Rd = Ra - \frac{(dN)}{(dt)}$$

where *Ra* is the rate of appearance (μmol/kg•min) and *Rd* the rate of utilization (μmol/kg•min). N = P•V•G, where *P* is the pool fraction (0.65), *V* the volume of distribution (ml), and *G* the blood glucose (μmol/ml). *F* is the rate of infusion of stable

isotope (μmol/min), *d* the delta and *t* the time (minutes). The glucose metabolic clearance rate was calculated as the quotient of *Rd* and the arterial glucose concentration.

Rates of glucose appearance represent final values after subtraction of exogenous dextrose infusion from the Biostator in protocols 3 and 4.

B. Gluconeogenesis:

Conversion of alanine to glucose was traced by a constant infusion of L-[1,2,3-^{13}C]-alanine (Merck Sharp and Dome, Pointe Claire, Québec, Canada) administered from 0 to 180 minutes. Gluconeogenesis was measured by a double stable isotope technique using L-[1,2,3-^{13}C]-alanine as the tracer to follow the conversion of ^{13}C-alanine to ^{13}C-glucose and D-[2,3,4,6,6-$^{2}H_5$]-glucose to measure the production of ^{13}C-glucose (Martineau et al., 1985). Alanine was chosen as the gluconeogenic substrate precursor because it is the major gluconeogenic amino acid extracted by the liver and constitutes with lactate the bulk of the gluconeogenic precursors (Ruderman, 1975; Dunn et al., 1976; Chiasson et al., 1977). This choice was also based on the possible release of lactate by the liver under certain circumstances and also on the possible removal of lactate through direct oxidation by the skeletal muscle during exercise and in the early recovery period (Brooks, 1986; Stanley et al., 1988; Gladden, 1989). ^{13}C-alanine was administered at a constant infusion rate of 4 mg/min resulting in a stable isotopic enrichment of 6 to 8% by 15 minutes of infusion, suggesting that an equilibrium had been reached within the alanine pool. This resulted in a stable ^{13}C-glucose enrichment of 0.5% after 30 minutes of the ^{13}C-alanine infusion.

Exact infusion rates were determined for each single experiment by measuring the isotope concentration in the infusate (D-[2,3,4,6,6-$^{2}H_5$]-glucose and L-[1,2,3-^{13}C]-alanine) using the mass spectrometry (Hewlett Packard model 5890 (GC) and 5970 (MS)) and by multiplying by the calibrated infusion rate.

Gluconeogenesis was calculated by the method validated by Chiasson et al. (1977) for steady and unsteady states condition for radioactive isotopes. The formula for ^{13}C-glucose appearance was as follow:

$$Ra\,^{13}C-glucose=\frac{d^{13}C-glucose}{dt}\times P\bullet V+Rd\times{}^{13}C-glucose$$

Gluconeogenesis was then calculated as follow:

$$\frac{Ra\,^{13}C-glucose}{ef\,^{13}C-alanine}$$

where *Rd* is the rate of disappearance ($\mu mol \cdot kg^{-1} \cdot min^{-1}$); *P* the pool fraction (0.65); *V* the volume of distribution (ml); *ef* the enrichment factor; *d* the delta; and *t* the time (minute). Alanine turnover was calculated by dividing the rate of infusion of ^{13}C-alanine by the enrichment factor of ^{13}C-alanine from which we subtracted the rate of infusion of ^{13}C-alanine.

Because of the isotopic dilution in the hepatic oxaloacetate pool, which allows a diversion of the labeled carbon from alanine, the methodology provides an underestimation of the net rate of conversion of alanine to glucose (Katz, 1985). This underestimation is brought by the diversion of the labeled carbon from alanine causing an overestimation of CO_2 production, and has been calculated to be approximately 2.0 (Hetenyi et al., 1982). Chiasson et al. (1977) have already discussed the limitations of the technique. This technique does not allow us to quantify gluconeogenesis, but does allow to measure relative changes under various conditions. Therefore, the data generated by this method are used only as an index of gluconeogenesis. In this study our results are expressed as percentage of the resting value.

C. Other Analyses

D-d_5-glucose and ^{13}C-alanine were separated from lactate and amino acids by column chromatography (Kreisberg et al., 1972). After lyophilization, the samples for D-d_5-glucose were resuspended in methanol and the isotope was then measured by gas chromatography-mass spectrometry (GC MS) under electron impact in the mode of selected ion monitoring (Hewlett Packard model 5890 (GC) and 5970 (MS)). D-d_5-glucose was analyzed at its 6-acetyl-[1,2:3,5]-bis-butaneboronyl-α-D-glucofuranose derivative (Weicko and Sherman, 1976) and ions at *m/z* 297 and 302 were measured. D-[1,2,3-$^{13}C_3$]-glucose was processed as D-d_5-glucose and measured at *m/z* 300. Samples for ^{13}C-lactate and ^{13}C-alanine were dissolved in methanol and analyzed by GC MS as their bisterbutyldimethylsilyl-trifluoroacetamide derivatives; ions at *m/z* 261, 264, 260 and 263 were monitored for each compound respectively. The choice of the tracer was done on the basis of a minimal overlap between the ions of interest and those arising from natural isotopic abundance taking also into account as much as possible carbon recycling and loss of label through futile cycles (Bier et al., 1973; Dunn et al., 1976; Kahlan et al., 1980; Tserng and Kalhan, 1983).

After deproteinization with 6% PCA, plasma glucose was analyzed by the hexokinase method, and plasma hydroxybutyrate, lactate, alanine, and glycerol were measured enzymatically using a Beckman spectrophotometer (Hohorst, 1965; Williamson, 1965; Bergmeyer, 1974; Cuthbert et al., 1978; Wieland, 1981). Plasma FFA were measured by a calorimetric method NEFA-C (Wako Pure Chemical Industries, LTD). Plasma for glucagon analysis was placed in prechilled tubes containing 50 µl of aprotinin Trasylol 10 000 K.I.U./ml (Miles #817113). Plasma insulin and glucagon were measured by radioimmunoassay (Biodata, NCS Diagnostics Inc.). For the glucagon assay, the antibody was specific for the pancreatic glucagon without any significant cross-over with the gastrointestinal hormones. The coefficient of variation of the assay was

below 5% and the limit of the assay was 30 pg/ml. For the insulin assay, the coefficient of variation was also below 5% and the lower limit was 5 µIU/ml or 36 pmol/L. Cortisol was measured with a radioimmunoassay using magnetizable particle (NCS Diagnostics Inc.) and growth hormone (Gamma -βCTGH #AA-67) with an immunoradiometric assay using monoclonal antibody coated tubes (NCS Diagnostics Inc.).

D. Statistical Analysis

Data are expressed as means ± SEM. Since all data measured were relatively stable over the second hour of exercise for all protocols, we have chosen to express them as a mean over that exercise period. The underneath table shows the time-course data for the protocol 2, the exercise control protocol. Therefore, for statistical purposes, the data presented here were computed for the last hour of the exercise period (from time 60 to 120 minutes). Since all subjects did not participated in all 6 protocols, each experiment is considered as a different subject for analytical purposes. Therefore, statistical comparisons were made using one-way analysis of variance (ANOVA) and differences between experimental groups were determined using t-test with Bonferroni correction for multiple testing at a 0.05 level of statistical significance (Kleinbaum et al., 1988). However, analysis of variance (ANOVA) using repeated measures were also done for the three (3) subjects who completed all six protocols. Significances were the same for both types of analysis.

TIME COURSE DATA
PROTOCOL 2: EXERCISE CONTROL (N=4)

	TIMES (minutes)							
Parameters	**0**	**60**	**70**	**80**	**90**	**100**	**110**	**120**
Plasma glucose (mmol/L)	4.46±0.04	4.57±0.06	4.53±0.11	4.40±0.13	4.32±0.17	4.22±0.17	4.19±0.14	4.20±0.13
Hepatic glucose production (µmol/kg•min)	12.1±0.2		23.2±1.7	24.8±1.8	27.8±2.5	29.9±3.7	30.3±3.5	28.5±3.5
Glucose utilisation (µmol/kg•min)	12.2±0.2		25.4±2.1	26.6±2.0	28.6±2.7	30.3±2.9	30.5±3.5	29.9±3.1
Gluconeogenesis (µmol/kg•min)	0.67±0.02		0.75±0.02	0.80±0.09	0.91±0.17	1.02±0.19	1.12±0.13	1.04±0.06
Plasma alanine (mmol/L)	0.21±0.02		0.26±0.01	0.26±0.01	0.25±0.01	0.24±0.01	0.24±0.01	0.23±0.01
Alanine turnover (µmol/kg•min)	3.2±0.1		7.9±0.2	7.7±0.4	7.2±0.5	7.4±1.3	7.5±1.2	7.5±0.9