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GLUCOSE AND GLYCOGEN METABOLISM DURING PROLONGED SWIMMING AND RECOVERY IN RATS

Colleen Ryan

Thesis submitted to the School of Graduate Studies and Research

in partial fulfillment of the requirements

for the degree of

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ABSTRACT

In order to assess glucose dynamics and the pathways (direct and gluconeogenic) by which muscle and liver glycogen is synthesized during and following prolonged submaximal exercise, twelve-hour-fasted catheterized rats were infused intravenously with [^{3}H-6]-glucose (or [^{3}H-3]-glucose) and either *i)* $NaH^{14}CO_3$, *ii)* [^{14}C-U]-lactate or *iii)* [^{14}C-1]-glucose (three gluconeogenic tracers) throughout two-hour basal, four-hour swim (or rest) and three-hour postexercise recovery periods. During recovery, rats were infused with either saline, lactic acid or glucose. Arterial blood samples were taken throughout the protocols. Liver and three muscles (soleus, white and red gastrocnemii) were excised and assessed for total and radiolabelled glycogen content. The results indicate that: *1)* While plasma glucose concentrations are maintained during the prolonged swim owing to matched doubling of both glucose production (R_a) and clearance (MCR), glucose increases during the recovery period as the fall in R_a lags behind that of MCR and the rate of gluconeogenesis remains elevated. *2)* Despite increases in direct glycogenesis during prolonged swimming, glycogen is significantly depleted in white and red gastrocnemii. Glyconeogenesis contributes at least 20-25% to glycogen repletion during fasted recovery. The source of this gluconeogenic substrate appears to be lactate which is formed locally in the muscles (as traced by recycled ^{14}C-glucose). Although not required to elicit muscle gluconeogenic activity, high lactate levels enhance glyconeogenesis, especially in the postexercise period. *3)* There is a high turnover of soleus glycogen at rest. The soleus does not respond to prolonged swimming but rather to the availability of

substrate (glucose) to synthesize its glycogen almost entirely via the direct route.

4) Glyconeogenesis is the primary pathway of hepatic glycogen synthesis. While glucose infusion is the most potent stimulus of glycogenesis, the combined effects of lactate and exercise appear synergistic in the formation of liver glycogen.

DEDICATION

I dedicate this work to the three Persons whose Grace made it possible - the Father, the Son and the Holy Spirit.

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I: THE PROBLEM: MUSCLE GLYCONEOGENESIS?

INTRODUCTION

The quest for the resolution of the pathways, mechanisms, sources and regulation of muscle glycogen synthesis is rooted in the crucial role glycogen plays in muscular contraction, and hence, movement. Glycogen, the storage form of glucose in tissues linked together by α-1-4- and α-1-6-linkages, is a critical source of fuel for muscular contraction. It is broken down by the action of glycogen phosphorylase upon hormonal stimulation (epinephrine) to glucose-1-phosphate which is then metabolized through glycolysis and the Krebs cycle for the production of energy in the form of ATP required for muscular contraction. Work performance and exercise duration have been shown to be directly correlated with the amount of glycogen in the muscle (1, 12, 52, 63, 82, 98). The pathway or pathways by which this critical energy source is repleted following exercise, however, have not been completely elucidated. Whether muscle glycogen is synthesized entirely from plasma glucose or partially from glucogenic precursors is the fundamental question of this thesis.

DEFINITION OF TERMS

Because the vocabulary associated with glycogen and carbohydrate metabolism in skeletal muscle may be misused in the literature and subtle distinctions of terminology are crucial to the understanding of this topic, the following terms will be

defined as used in the context of this thesis. *Gluconeogenesis* is the formation of *glucose* from noncarbohydrate sources such as lactate, glycerol, alanine and glutamine. It takes place in the liver and kidney cortex. While renal glucose production and release into plasma occurs primarily in prolonged starvation, hepatic gluconeogenesis serves to maintain blood glucose levels independent of dietary intake and to convert lactate generated in exercise into glucose (21). Because muscle lacks the enzyme glucose-6-phosphatase (80) which liberates glucose into the plasma, muscle is not capable of gluconeogenesis in the *strict* sense of the meaning (i.e. formation of *glucose*). *Glycogenesis* refers to the synthesis of glycogen by any route while *glyconeogenesis* (the topic of this thesis) is defined as the formation of tissue glycogen from precursors other than plasma carbohydrates. In the context of this thesis, glyconeogenesis is also referred to as the *gluconeogenic formation of glycogen* as glyconeogenesis and gluconeogenesis are essentially the same pathway except that in glyconeogenesis, glucose-6-phosphate is converted into glycogen as the final product rather than being liberated into the plasma as glucose. This pathway is also referred to as the *indirect pathway* of glycogen synthesis, although some use this term specifically in reference to the pathway, glucose → 3-carbon products → glycogen (glucose). (Refer to pp. 3-4 for Figures I.1 and I.2 of the proposed glyconeogenic pathways in liver and muscle, respectively, and enzymes involved.)

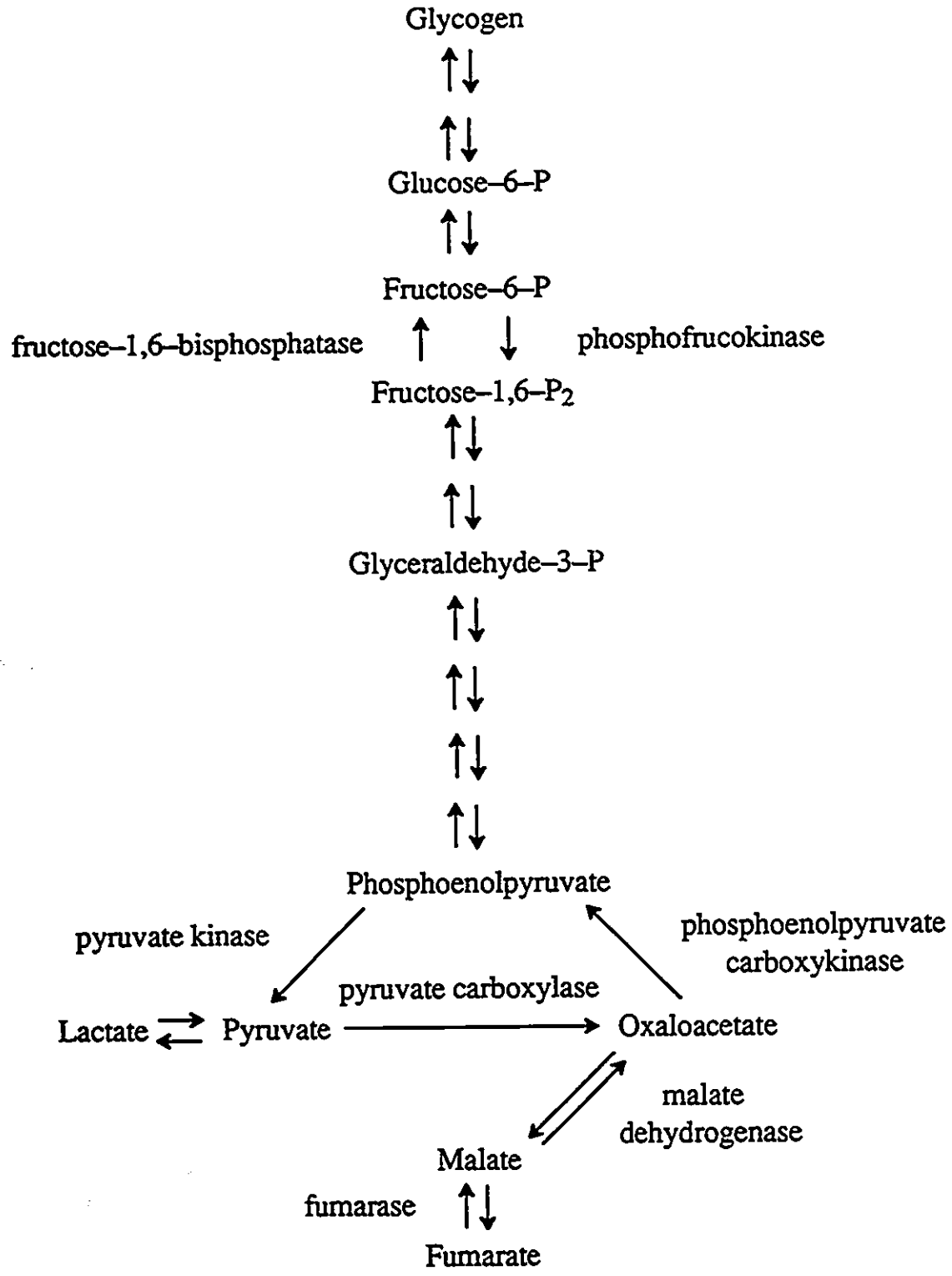

Figure I.1: Schematic of glyconeogenic pathway in liver.

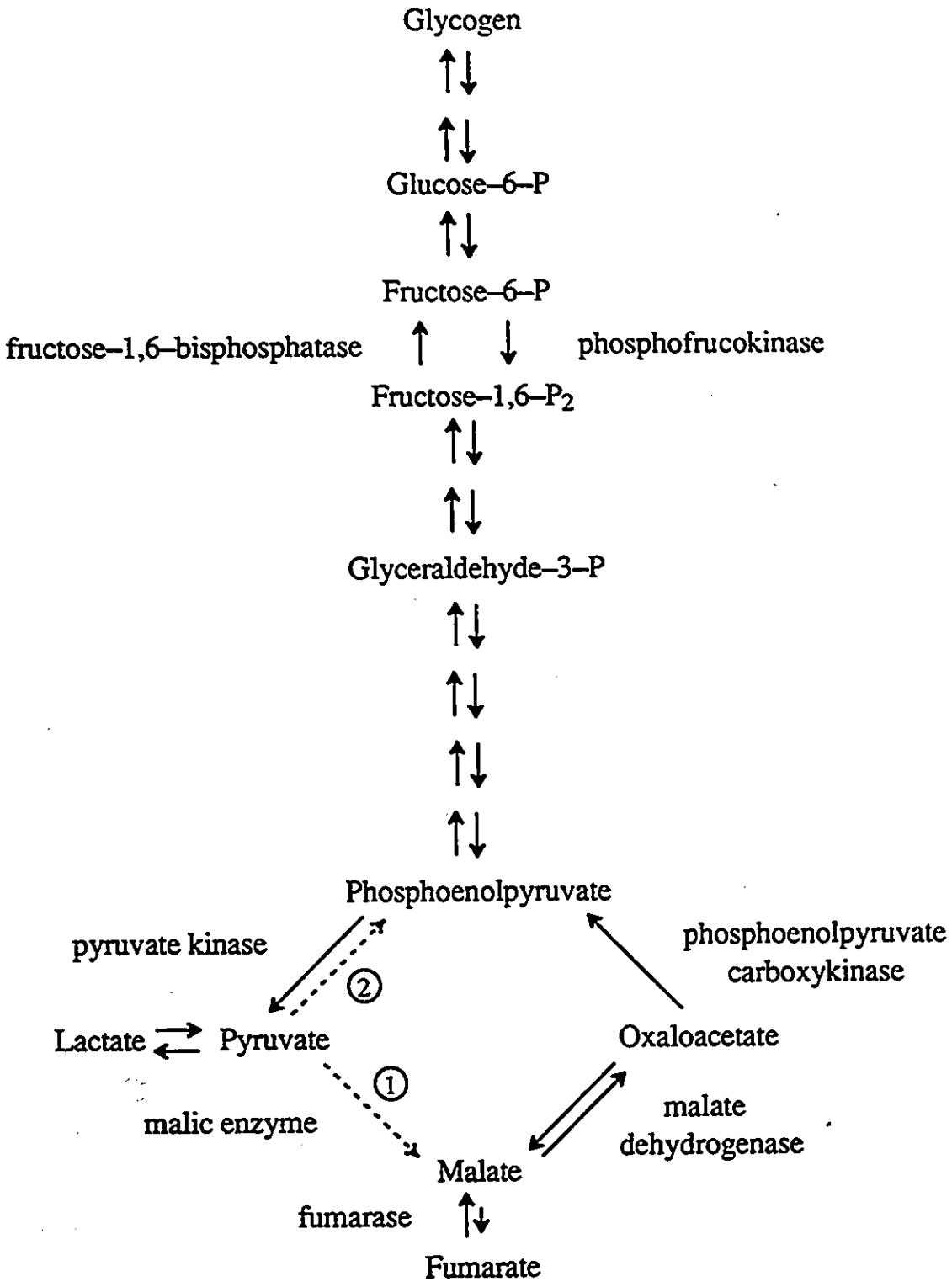

Figure I.2: Schematic of proposed glyconeogenic pathway in muscle.

HEPATIC GLYCOGENESIS

Prior to 1980, it had been generally accepted that hepatic glycogenesis occurred by the direct uptake of glucose from plasma (i.e. glucose → glucose-6-phosphate → glycogen) during refeeding following a fast. However, glucose itself, even in high concentrations and in the presence of insulin, is a poor precursor for hepatic glycogen synthesis *in vitro* (see references cited in ref. 79). This "glucose paradox" (as it has since become known) was also noted in perfused rat liver preparations in which glycogen was formed primarily from gluconeogenic precursors rather than glucose (51). Following ingestion of a glucose-rich meal labelled with [^{14}C-U]- and [^{3}H-6]-glucose, the gluconeogenic rate in mice was high and half of the glycogen seemed to be formed from a glucose-6-phosphate pool which was never labelled (9), again suggesting that hepatic glycogen was formed from precurors other than glucose. In his studies in man, Radziuk has shown that during oral glucose loading following fasting, less than 10% of the glucose is taken up on its first pass through the liver (92) and that while 10 g of hepatic glycogen was made directly from glucose (87), at least 15 g of glycogen was synthesized from gluconeogenic precursors (86, 88).

This indirect or glyconeogenic pathway for hepatic glycogen formation (glucose → lactate → glucose-6-phosphate → glycogen) was also confirmed by Newgard et al. (78, 79) and Scofield (99) using infusions of double labelled glucose ([^{3}H-3]- and [^{14}C-U]-glucose) into rats along with a glucose load and assessing the ^{3}H/^{14}C ratios in hepatic glycogen. If glycogen was formed directly from glucose, then

the specific activities of glucose in glycogen would be the same as those of the infused glucose. If, however, glucose was converted to lactate prior to glycogen formation (indirect pathway), then the tritiated label would be lost in glycolysis, resulting in a lower ratio of $^{3}H/^{14}C$. Indeed, the ratios of labelled glucose in glycogen were no more than 37% of that in the infused glucose (78), suggesting significant glyconeogenic activity in the liver.

MUSCLE GLYCONEOGENESIS

The Original Question - Is It Possible?

While it is fairly well accepted now that, during refeeding following a fast, liver synthesizes at least 50% of its glycogen via the indirect or glyconeogenic route (45, 65, 69, 70, 71, 75, 78, 79, 86, 88, 89, 90, 91, 99, 100), the question is, however, whether skeletal muscle is capable of such glyconeogenic activity or rather, as classically accepted, muscle glycogen is synthesized strictly or primarily from the uptake of plasma glucose. This question remains incompletely resolved after almost 70 years of investigation.

Historically, it was generally accepted that muscle glycogen was synthesized solely from glucose and that conversion of lactate to carbohydrate could occur only in liver. This was based on a number of early investigations in the 1920's and 1930's which failed to confirm the findings of Meyerhof - the apparent conversion of lactate to glycogen in perfused hindlimbs of frogs (76, 77). He claimed that the majority of lactate in muscle was reconverted to glycogen within the muscle and only about 20%

of the lactate was oxidized to CO_2. Earlier, Elias and Schubert (see ref. 24) had detected no increases in muscle glycogen following intra-arterial injection of lactic acid into hindlegs of dogs. In 1925, perfusions of dog hindlimbs with DL-lactate by Janssen and Jost also failed to show any increases in muscle glycogen (see ref. 24). Eggleton and Evans (41) found no increases in muscle glycogen in cats following injections of large amounts of lactate. They also repeated Meyerhof's frog hindlimb perfusions and found only very minimal quantities of glycogen were formed, apparently not above control levels. In 1935, Sacks and Sacks (97) concluded that there could not have been any detectable muscle glycogenesis from lactate in rabbit muscles following electrical stimulation based on the observation that the total amount of carbohydrate loss exceeded the disappearance of lactate during the recovery period. In 1932, Long and Horsfall (see ref. 24) claimed that infusions of 2-10% lactic acid solutions produced no significant increases in muscle glycogen in decapitated eviscerated cats. Twenty years later, perfusion with isotopically labelled lactate ([^{13}C-1]- or [^{13}C-2,3]-lactate) produced no ^{13}C-labelled glycogen in heart muscle (see ref. 24).

In the mid 1960's, the emphasis on evidence for or against muscle glyconeogenesis shifted towards the presence or absence of the necessary enzymes. Although Krebs and Woodford (68) demonstrated the presence of significant activity of fructose-1,6-bisphosphatase (FBPase) in muscle, they maintained that muscle was incapable of synthesizing glycogen from lactate and any labelling of muscle glycogen following administration of isotopically labelled lactate was due to an isotopic-

exchange reaction in muscle (presumably at the level of pyruvate kinase) rather than to net synthesis of glycogen from lactate. The authors did, however, propose that glycogen synthesis from phosphorylated three-carbon compounds (α-glycerophosphate and phosphoenolpyruvate) was possible in light of the presence of FBPase activity. In 1967, Opie and Newsholme (80) not only confirmed the presence of significant FBPase activity in muscle, but also demonstrated high activities of phosphoenolpyruvate carboxykinase (PEPCK) and malic enzyme in muscle. They also reported very low activities of both glucose-6-phosphatase and pyruvate carboxylase in muscle (80). Due to the lack of pyruvate carboxylase activity, they too dismissed the possibilty of muscle glyconeogenesis from lactate and suggested that the high activities of FBPase and PEPCK in muscle were to ensure levels of α-glycerophosphate and oxaloacetate (OAA) did not accumulate during exercise so that the α-glycerophosphate - DHAP (dihydroxyacetone phosphate) and malate - OAA cycles would not be inhibited during postexercise recovery (80). Thus, by this time, almost fifty years later, there was virtually no corroborated evidence to confirm Meyerhof's original claim of muscle glyconeogenesis and it was generally accepted that conversion of lactate to carbohydrate could occur only in liver and kidney.

The controversy was given new life, however, in 1970 by Bendall and Taylor (11) who repeated Meyerhof's original experiments and demonstrated *in vitro* glycogen synthesis from lactate in frog satorius muscles. Although they showed a high correlation between the rate of lactate disappearance and the rate of glycogen formation, demonstrating a possible link between the two and speculated on potential

pathways of such glycogen synthesis, they did not provide direct evidence supporting the conversion of lactate to muscle glycogen. Moreover, these experiments were performed in excised amphibian muscles which may not be applicable to the postexercise situation in human muscle.

Hermansen and Vaage (53), however, extended the question to exercising humans. Their approach was to determine whether the rate of lactate efflux from muscle was sufficient to explain the rate at which muscle lactate diminished and to account for the muscle glycogen formed during the recovery period following maximal intermittent exercise (cycling) in humans. By measuring leg blood flow, arterial-femoral venous differences and muscle glycogen concentrations, they presented a case for muscle glyconeogenesis based on the following evidence: *1)* there was rapid glycogen resynthesis during a fasted recovery period, *2)* only 3% of the glycogen synthesized could have been accounted for by the uptake of blood glucose, *3)* the disappearance of lactate and the formation of muscle glycogen coincided in time and in magnitude, *4)* neither lactate nor alanine efflux from the muscle could account for lactate disappearance nor for glycogen synthesized (via hepatic gluconeogenesis), *5)* glycolytic intermediates could not account for all glycogen synthesis, and *6)* glyconeogenic enzymes had been demonstrated in fast-twitch muscles (74, 80). Their case for muscle glyconeogenesis, however, was based on strong but indirect evidence. However, others (20) have questioned their experimental assessment of leg blood flow suggesting it may have been underestimated by 50%, and have criticized their lack of consideration of total body

lactate pools in their calculations.

In 1979, McLane and Holloszy (74) provided the first direct evidence of the conversion of lactate into muscle glycogen. They demonstrated not only significant increases in glycogen in fast-twitch muscles in rat hindlimbs perfused with lactate as the only substrate, but also the direct incorporation of ^{14}C-lactate into muscle glycogen. In addition, they demonstrated significant activities of malic enzyme, PEPCK and FBPase in the muscles. On the other hand, the soleus muscle, which is comprised primarily of slow-twitch red fibers, showed relatively low glyconeogenic capability presumably due to the low activities of PEPCK and FBPase found in the muscle. These results, however, were obtained from perfused hindlimbs in rats that had been swum to exhaustion the previous day and maintained on a low carbohydrate diet overnight, and may not be applicable to the *in vivo* postexercise situation in human muscle. Despite the limitations in the experimental design, this was the first direct evidence that muscle was indeed capable of synthesizing glycogen from lactate at least *in vitro*.

Shiota et al. (101) confirmed the *in vitro* synthesis of lactate into glycogen in rat muscle. They perfused rat hindlimbs with [^{3}H-6]-glucose and [^{14}C-U]-lactate to assess the relative contributions of glucose and lactate to muscle glycogen synthesis. In muscles which were not depleted of their glycogen and in which the rates of glycogen synthesis were low, the contribution of lactate to glycogen formation exceeded that of glucose. In prestimulated muscles, however, in which glycogen was depleted by half, the incorporation of lactate into glycogen was less than that of

glucose but at high lactate concentrations (17 mM) was about equal to that of glucose. They also confirmed that muscle glyconeogenesis from lactate occurred in fast-twitch white and red fibers, but to a much lesser degree in slow-twitch red muscles. But again, these results were obtained in isolated rat hindlimbs which were prestimulated to deplete glycogen and perfused with artificial concentrations of glucose, lactate and insulin; the relevance to the postexercise synthesis of glycogen in human muscle thus remains questionable.

The Contemporary Question - Is It Physiologically Significant?

With the strong (indirect) case for muscle glycogen synthesis from lactate (53) and the actual *in vitro* demonstrations of the incorporation of lactate into rat muscle glycogen (74, 101), the emphasis of the controversy of muscle glyconeogenesis shifted from the question of its existence to its physiological significance and quantitative importance *in vivo*.

Brooks and Gaesser (20) examined the postexercise fates of lactate produced in rats run to exhaustion on a treadmill by injection of ^{14}C-lactate into the animals. In striking contrast to Hermansen and Vaage (53), they proposed that during recovery from exhaustive exercise, the majority of lactate was oxidized to CO_2 with only about 18% being converted to muscle glycogen. Based on radiochromatographic and indirect evidence, they also deduced that the majority of muscle glycogen was synthesized from glucose made via hepatic or renal gluconeogenesis (20). Radiochromatograms of liver and kidney showed large and rapid incorporation of ^{14}C-

lactate label into glucose while those from muscle indicated only small pools of radiolabelled glyconeogenic intermediates and much higher incorporation of the ^{14}C label in rats injected with ^{14}C-glucose than those receiving the ^{14}C-lactate tracer. Restoration of muscle glycogen parallelled that of blood glucose. In addition, while lactate removal following exercise was rapid, the amount of glycogen formed during this time was small. Finally, if all of the lactate present at the end of exercise was converted to glycogen, it could account for only 40% of the glycogen synthesized (20). They concluded, therefore, that although lactate was converted to muscle glycogen, the pathway was likely primarily via hepatic (and renal) glucose formation.

These conclusions should be considered in light of the inherent limitations in the experimental design. Firstly, direct incorporation of ^{14}C-lactate into muscle glycogen as distinct from that of ^{14}C-glucose was not demonstrated, *per se*. That the ^{14}C label in muscle glycogen following postexercise injection of ^{14}C-lactate arose primarily from ^{14}C-glucose formed via hepatic gluconeogenesis was based on indirect evidence (above). Thirdly, the incorporation of ^{14}C-lactate into muscle glycogen resulting from injection of the tracer into the blood may not adequately label intramuscular lactate contributions to glycogen synthesis and may only represent those of blood lactate which may not be a primary source of muscle glycogen synthesis. Finally, the radiochromatographic results as well as the indirect evidence may not accurately *quantitate* intramuscular lactate contributions to muscle glycogenesis.

In contrast to Brooks and Gaesser (20), but in quantitative agreement with Hermansen and Vaage (53), Åstrand et al. (7) proposed that the major postexercise

fate of lactate in humans is glyconeogenesis and that the dominant substrate for muscle glycogen is indeed lactate. This was based on calculations derived from measurements of lactate, oxygen consumption, CO_2 expiration, blood flow, blood glucose, muscle glycogen, lactate and hexosemonophosphates before and after heavy exercise and recovery in humans. They estimated that, of the lactate formed during the exhaustive exercise, 10% was taken up by the liver, 40% was oxidized and the remaining 50% of the lactate was assumed to have been for used in the formation of muscle glycogen. Moreover, the potential conversions of muscle hexosemonophosphates, free glucose and glycerophosphates as well as blood glucose to glycogen could not account for all of the glycogen synthesized during the recovery period, again supporting a strong case for muscle glyconeogenesis. Unfortunately, their case is based purely on indirect evidence.

Although the study by Newgard et al. (78) focused on the pathways of hepatic glycogen synthesis, they deduced that muscle is a relatively nonglyconeogenic tissue and that glycogen synthesis occurs primarily, if not entirely, via the direct uptake of plasma glucose. This was based on the observation that, with [^{14}C-1]-glucose infusion into rats, most of the ^{14}C label in muscle glycogen was retained in the C-1 position, indicative that the tracer was taken up directly from plasma into muscle glycogen with little or no recycling through the glyconeogenic pathway. This conclusion, however, was based on the percentages of ^{14}C in the C-1 position in leg muscles of only four rats and, moreover, these percentages varied from 75% to 90% which may suggest that up to 25% of glycogen may have been synthesized via the glyconeogenic route.

In addition, there was no consideration of the different types of muscles which have been shown to demonstrate varying capacities for glyconeogenesis (15, 62, 74). Finally, it should be noted that these studies were conducted in resting animals rather than following exhaustive exercise (as in the studies described above) in which muscle glycogenesis is expected to be stimulated (eg. ref. 26).

The Pathway

Another indirect approach to the question of muscle glyconeogenesis has been the elucidation of the potential pathways by which it could occur. The pathway by which lactate could be converted to glycogen in the muscle remains obscure. (For this discussion, refer to pp. 3-4 for Figures I.1 and I.2, the schematics of the proposed glyconeogenic pathways in liver and muscle, respectively.) Because the equilibrium constant of pyruvate kinase, the enzyme which converts phosphoenolpyruvate (PEP) into pyruvate, is 6.5×10^3 (see ref. 40) and due to its high positive standard free energy, the reaction is generally considered irreversible. In the liver, therefore, the formation of PEP from pyruvate takes place via an enzymatic bypass. Pyruvate in liver mitochondria is converted to oxaloacetate (OAA) via pyruvate carboxylase. OAA then enters the cytosol by the reversible transamination to aspartate, where it undergoes decarboxylation by phosphoenolpyruvate carboxykinase (PEPCK) to form PEP. Since the glycolytic enzyme, phosphofructokinase (PFK), is also irreversible, the last of the key gluconeogenic enzymes, fructose-1,6-bisphosphatase (FBPase), catalyzes the

conversion of fructose-1,6-bisphosphate to fructose-6-phosphate on the way to hepatic glycogen (or glucose). Although fast-twitch muscle fibers exhibit significant activities of PEPCK and FBPase (31, 80), they apparently do not have significant pyruvate carboxylase activity (80) and, therefore, muscle lactate cannot be converted to glycogen by the same pathway as in the liver.

In light of the absence of pyruvate carboxylase in muscle, two alternate pathways of muscle glyconeogenesis have been proposed to account for the conversion of lactate into glycogen. Significant activities of extramitochondrial malic enzyme (NADP-dependent) as well as phosphoenolpyruvate carboxykinase, however, have been reported in rat and mouse muscles (31, 74, 80, 109). Pyruvate, then, could be carboxylated via malic enzyme to form malate in the cytosol which would be converted to OAA by way of malate dehydrogenase as first suggested by Bendall and Taylor (11); OAA, in turn, could be decarboxylated by PEPCK to form PEP (proposed pathway *1* in Figure I.2, p. 4) Support for this route has come from the demonstration of significant inhibition of lactate incorporation into glycogen in perfused frog muscle by 3-mercaptopicolinic acid, a potent inhibitor of PEPCK (28). In addition, the author suggested that the source of reducing power necessary for malic enzyme activity would come from transhydrogenase reactions to form NADPH from the NADH produced via lactate dehydrogenase. In recent studies, Talmadge and Silverman (108, 109) have demonstrated not only that chronically active gastrocnemius muscles in C57B1/6J dy^{2J}/dy^{2J} mice undergo glyconeogenesis at rates four times those of normal muscles, but also significantly increased activities of malic

enzyme, malate dehydrogenase and PEPCK in these muscles with abnormally high glycogen concentrations.

Shiota et al. (101) have pointed out, however, that although the activity of muscle malic enzyme is high (80), the decarboxylation reaction is favoured and there is no evidence for significant formation of NADPH in muscles. They have also reported no inhibition of glycogen synthesis from lactate in perfused rat hindlimbs by mercaptopicolinic acid (101), which is not consistent with this proposed pathway. Pagliassotti and Donovan (81) have recently corroborated the lack of inhibition by mercaptopicolinic acid on glycogen synthesis and have concluded that the glyconeogenic route in rabbit muscle, at least, is extramitochondrial and does not involve PEPCK.

Dyson et al. (40) have provided evidence that, despite its high equilibrium constant favouring the formation of pyruvate from PEP, muscle pyruvate kinase is capable of synthesizing PEP at rates which would support the reported levels of glyconeogenesis, thus resolving the apparent lack of an enzymatic bypass and possibly accounting for the levels of the enzyme in muscle which surpass that required for the reported rates of glucose catabolism (proposed pathway 2 in Figure I.2, p. 4). However, no direct evidence supporting either of these two proposed glyconeogenic pathways in muscle exists to date and, thus, the issue of how lactate is converted into glycogen in muscle remains obscure.

THE PROBLEM

Up until 1986, then, at the onset of the investigations for this thesis, direct evidence in favour of muscle glyconeogensis in physiological conditions was scarce and contradictory, despite the number of investigations supporting its potential role and proposed pathways in glycogen repletion especially following exercise. It was our primary intention, then, to provide direct *in vivo* evidence in favour of or against glyconeogenesis as a significant contributor to muscle glycogen repletion following exercise without specifically addressing the underlying mechanisms, regulations and pathways by which it may occur.

II: THE APPROACH TO THE PROBLEM

THE RATIONALE

The physiological significance of muscle glyconeogenesis remains incompletely resolved. At the time of the beginning of the investigations for this thesis (1986), the few *in vivo* studies supporting muscle glyconeogenesis as a significant contributor to glycogenesis (7, 53) had examined glyconeogenic activity during the postexercise period following intense or exhausting exercise. Such intense exercise is characterized by very high lactate concentrations both in plasma (up to 20 mM) and muscle (up to 26 mmol·kg^{-1} wet weight muscle) (53). In addition, the *in vitro* studies which demonstrated muscle glyconeogenesis (directly) did so in the presence of very high lactate concentrations in the perfusate (74, 101). Moreover, Newgard et al. (78)

demonstrated insignificant glyconeogenic activity in rested rat muscle (i.e. glycogen-rich state with low lactate levels). By 1986, then, the only evidence (direct or indirect) supporting glyconeogenesis came from experimental models which were characterized by the presence of very high lactate concentrations, suggesting that perhaps high lactate levels are required to elicit muscle glyconeogenesis.

Indeed, studies which emerged following the onset of our investigations further supported this notion of the requirement for high lactate levels in order to elicit glyconeogenic activity in muscle. Stevenson et al. (107) demonstrated glycogen formation from lactate in perfused rat hemicorpus (which had been previously depleted of its glycogen via electrical stimulation and exposure to epinephrine) but only in the presence of high lactate levels (10.5 and 18 mM). They suggested that glyconeogenic activity could conceivably be lower following training owing to increased lactate clearance from the blood (37), and hence, less substrate would be available to the muscle for glyconeogenesis (107). Johnson and Bagby (62) demonstrated the direct *in vivo* incorporation of ^{14}C-lactate (as distinct from that of ^{14}C-glucose arising from hepatic gluconeogenesis) into muscle glycogen during recovery from exhaustive treadmill running in rats but only in white gastrocnemius muscle and only in the presence of elevated plasma lactate levels produced by exogenous infusions of either lactate or glucose. This potential requisite for high substrate availability to induce significant muscle glyconeogenesis can also be inferred from the direct demonstration of lactate incorporation into glycogen in the chronically active *dy*^{2J} gastrocnemius muscle in C57B1/6J *dy*^{2J}/*dy*^{2J} mice (108, 109). This muscle

is characterized not only by increased glycogen content but also by elevated lactate levels (approximately 8 mM) (108). The rates of glyconeogenesis in these muscles were almost four times those of normal muscles (108). These three studies, then, underline the capability of muscle to synthesize glycogen via the glyconeogenic route, yet still question whether it can occur at physiologically significant rates in physiological conditions (i.e. low lactate levels); in retrospect, they also further support our initial approach to the problem (to follow).

THE APPROACH

Exhaustive exercise models are the most attractive in examining the question of the *in vivo* physiological significance of muscle glyconeogenesis since they are characterized by significant muscle glycogen depletion (eg. refs. 46, 53, 62) as well as preferential (relative to liver, refs. eg. 44, 46) and rapid repletion even in the absence of exogenous substrate (eg. refs. 46, 53, 62). This is due perhaps to the stimulation of glycogen synthase by the very low postexercise concentrations of glycogen (26). However, as many of these exercise models are of moderate to high intensity, they produce high levels of lactate in plasma and muscle (eg. ref. 53), making it difficult to distinguish the effects of exercise, *per se*, (in terms of associated changes in enzymatic activities and regulation, heightened energy production etc.) from those of the increased availability of gluconeogenic substrate (lactate) itself in the stimulation of the glyconeogenic pathway in the postexercise repletion of muscle glycogen.

Prolonged submaximal exercise (such as prolonged swimming), on the other hand, is characterized not only by significant muscle glycogen depletion (43, 44) and *minimal* lactate accumulations in plasma and muscle (43), but also by significant glycogen repletion during fasted postexercise recovery periods (43, 44). Therefore, while the stimulus for increased muscle glycogen synthesis (i.e. prior exercise) is maintained, the effects of lactate can be separated out. In addition, such a model allows for the postexercise introduction of lactate (via exogenous infusion) in order to examine (to some extent) the relative roles of systemic *versus* locally formed (intramuscular) lactate in glyconeogenesis. Thus, we have chosen a rat exercise model in which the animals swim for a prolonged period (four hours) and recover for three hours either in a fasted state or with infusions of exogenous lactate in order to study the effects of *i)* exercise, *per se*, (and the contributions of locally formed lactate), *ii)* the availability of (systemic) substrate, *per se*, (by infusion of exogenous lactate into rested animals), and *iii)* the combination of both exercise and substrate (by postexercise infusion of lactate), in the stimulation of muscle glyconeogenesis.

Three specific muscles - the soleus, white gastrocnemius and red gastrocnemius - were chosen for the examination of glyconeogenesis based on their metabolic profiles and previously reported glyconeogenic capacities. As each represents the "purest" form of the fiber content which makes up the majority of the fibers in the muscle (4, 5), many studies examining glycogen metabolism in specific muscles (eg. refs. 60, 62, 74, 113) have used these three, thus allowing comparisons of our data to that in the literature. The soleus muscle is comprised primarily

(approximately 85%, refs. 4, 5) of slow-twitch red fibers also known as SO or type I fibers. They have a high respiratory capacity, are highly dependent on the aerobic oxidation of both carbohydrate and free fatty acids (FFA) for energy and have low glycogenolytic and myosin ATPase activities (83). The white gastrocnemius muscle is made up primarily (84%) of fast-twitch white fibers (FG or type IIb) (5) which are very highly glycolytic and have low respiratory capacities (83). They are also highly glycogenolytic and have high myosin ATPase activity. Finally the red gastrocnemius is comprised of approximately 62% fast-twitch red fibers (FOG or type IIa) (5). These fibers are both glycolytic and oxidative in nature with high respiratory and glycogenolytic capacities as well as high myosin ATPase activity (83). Fast-twitch red fibers also have higher concentrations of mitochondria and myoglobin than white fibers. The metabolic characteristics of these fiber types may reflect different metabolic tendencies not only during exercise, but also in the postexercise recovery period, specifically with regard to glyconeogenesis (15, 62, 74). Although all three muscles are reportedly depleted in prolonged swimming and experience significant postexercise glycogen synthesis (26, 43, 111, 113), we found that the soleus glycogen is not depleted during prolonged swimming in our exercise model, and thus becomes useful as an "inactive control".

While the focus of these investigations is muscle glyconeogenesis, liver is included primarily as a "control" for our tracer methods detecting glyconeogenesis as it is well accepted that the gluconeogenic route contributes significantly to hepatic glycogenesis (45, 65, 69, 70, 71, 75, 78, 79, 86, 88, 89, 90, 91, 99, 100). Inclusion

of the liver in the studies also allows for the confirmation of the preferential repletion of muscle glycogen (relative to liver glycogen, eg. refs. 44, 46, 62) during a fasted postexercise recovery, as well an examination of the combined effects of lactate and exercise on hepatic glycogenesis.

While the effects of moderate and intense exercise on glucose turnover and gluconeogenesis (19, 38, 44, 61, 102, 104, 117, 123) have been well documented, less information is available on glucose dynamics during prolonged submaximal exercise. The rates of glucose production (R_a) and metabolic clearance (MCR) as well as hepatic gluconeogenesis (R_{ag}) have not been characterized during prolonged swimming and postexercise recovery in rats, allowing us the opportunity to first compare glucose turnover in prolonged submaximal exercise to that during more intense exercise before assessing the pathways of muscle glycogen repletion.

III: TRACER METHODOLOGY

INTRODUCTION

Radioactive tracers have been chosen as the preferred alternative for studying glucose and glycogen metabolism *in vivo* for a number of reasons. Firstly, measurements of metabolite concentrations alone give no indication of turnover rates; they simply determine whether a metabolite concentration is changing with time. Using tracer dilution as an indicator, systemic metabolite *fluxes* can be determined. Similarly, pathways can be tracked by determining the final destinations of labelled

carbons which started in a particular position of a particular substrate.

Generally, isotopic tracers are molecules in which one or more atoms are "labelled" by having an unequal number of protons and neutrons and can thus be used to "trace" pathways of metabolism. Isotopes may be stable (eg. ^{2}H, ^{13}C) or radioactive (eg. ^{3}H, ^{14}C) depending on the number of neutrons, allowing for subsequent quantitation of the labelled molecule using mass spectrometry or liquid scintillation counting, respectively. The underlying assumption in the use of metabolic tracers is that the labelled molecule will not be discriminated from but rather trace the movement of the unlabelled molecule.

TRACER METHODS AS APPLIED TO GLUCOSE METABOLISM

Steady State Kinetics

Just as the elucidation of the pathways of postexercise muscle glycogen repletion in this thesis begins with an evaluation of glucose turnover during and following exercise, the discussion of the methods begins by describing the principles of tracer methodology as applied to glucose metabolism, most of which are applicable to glycogen metabolism. Since the liver is the only site of significant glucose production (via glycogenolysis and gluconeogenesis) and release into plasma (with renal gluconeogenesis becoming important only in starvation, ref. 21), the rate of appearance of glucose in plasma, R_a, is the same as the rate of production. In physiological steady state, R_a is equal to R_d, the rate of glucose disappearance from plasma, so as to maintain a constant glucose concentration, C. It follows, then, that

the rate of appearance (i.e. infusion rate) of tracer glucose (eg. [^{3}H-6]-glucose) in plasma, which is denoted as R_a^*, is equal to its rate of utilization, R_d^*, to maintain a constant tracer concentration, C^*, in steady state. Then,

$$\frac{R_d}{R_d^*} = \frac{C}{C^*}$$

Rearranging the equation gives

$$R_d = \frac{R_d^* \times C}{C^*}$$

Since R_d equals R_a, and R_d^* equals R_a^*, experimentally, the rate of glucose production is calculated by

$$R_a = \frac{R_a^* \times C}{C^*}$$

The metabolic clearance rate of glucose (MCR) is the volume of blood or plasma cleared of glucose in one minute. It is defined by the equation

$$MCR = \frac{R_d}{C}$$

Since R_d/C equals R_d^*/C^* and since R_d^* equals R_a^*, then, experimentally, MCR is calculated by

$$MCR = \frac{R_a^*}{C^*}$$

Nonsteady State Kinetics

There are many *in vivo* physiological conditions in which the rate of production of glucose may not equal that of glucose removal, such as exercise. Under these conditions, glucose kinetics are frequently described using a one-compartment system:

$$V\frac{dC}{dt} = -MCR \cdot C + R_a$$

where V is the volume of glucose distribution (V = 20% of body weight) and the other parameters are as explained above. Since the tracer behaves analogously, it also obeys the above equations. Using these two equations, formulae were derived for glucose production and clearance (106):

$$R_a = \frac{R_a^*}{a} - \left[\frac{p \cdot V \cdot C}{a}\frac{da}{dt}\right] \qquad (I.1)$$

$$MCR = \frac{1}{C^*}\left[p \cdot V \cdot \frac{dC^*}{dt} - R_a^*\right] \qquad (I.2)$$

where C^* is the plasma concentration of labelled glucose, C is total plasma glucose concentration, R_a^* is the rate of infusion of tracer glucose and a is the specific activity of plasma glucose (C^*/C). p is a "pool fraction", usually 0.65-0.75, added to account for nonuniform glucose distribution.

The tracer chosen for the determination of total rates of glucose production and clearance in these studies is [^{3}H-6]-glucose. Because the label on the sixth carbon

is lost in the pyruvate carboxylase reaction and in the equilibration of oxaloacetate, malate and fumarate (39, 79, 101, 124) the labelled glucose cannot undergo further gluconeogenesis and thus recycle into the plasma once it is oxidized to lactate. Therefore, dilution of the specific activity of [^{3}H-6]-glucose in plasma is a true reflection of the production of glucose.

TRACER METHODS AS APPLIED TO GLUCONEOGENESIS

The conventional means of measuring the rate of gluconeogenesis is to determine the total rate of glucose production and the fraction of glucose arising from a ^{14}C-labelled precursor (124). The percent of glucose arising from the precursor is calculated as

$$\% \ glucose = \frac{SA\ (^{14}C\text{-}glucose) \times carbon\ correction}{SA\ (^{14}C\text{-}precursor)}$$

where *SA* is specific activity (C*/C) and *"carbon correction"* accounts for the different number of carbons in the precursor and glucose. The rate of gluconeogenesis from the precursor, then, is

$$rate\ of\ gluconeogenesis = \% \ glucose \times R_a$$

The choice as to the appropriate tracer as the labelled precursor to measure gluconeogenic formation of glucose (and glyconeogenesis) requires careful deliberation and an understanding of label dilution and exchange as detailed in the following sections.

Metabolic Exchange of Labelled and Unlabelled Carbons

Both the gluconeogenic pathway and the Krebs cycle must pass through the oxaloacetate (OAA) pool. Therefore, carbon atoms of gluconeogenic precursors (lactate, pyruvate, alanine) will mingle in the same OAA pool as carbons of acetyl CoA entering from the Krebs cycle. Consequently, on their way to glucose, ^{14}C-labelled carbon atoms of gluconeogenic precursors may be exchanged with ^{12}C atoms coming from acetyl CoA carbons, thereby lowering the specific activity in glucose and underestimating the gluconeogenic contributions to glucose production. Although carbons originating in acetyl CoA can be incorporated into glucose via this cross-over of metabolic pathways, it should be noted that acetate can make no net contributions to glucose production.

Hetenyi (54, 55) has proposed a correction factor, *H*, for the underestimation of gluconeogenic rates from some labelled precursors due to this metabolic exchange in the OAA pool. It is based on a one-to-one exchange of ^{14}C for ^{12}C atoms in the OAA pool and on the ratio of specific activities of the first and third carbons in glucose as a result of the infusion of [^{14}C-2]-acetate. This glucose-labelling ratio reflects the influxes of carbon atoms from acetyl CoA and other sources into OAA (119). *H* has been estimated at 2.2 and 1.55 in normal (nonexercising) dogs and rats, respectively (54, 55).

This correction factor has been calculated based on a number of assumptions, most important of which is that the only source of ^{14}C in glucose is due to incorporation from [^{14}C-2]-acetate or, at least, that incorporation of ^{14}C atoms from

other sources leads to the same pattern of label distribution in glucose as expected from [^{14}C-2]-acetate (55). However, alternative routes by which the label can be incorporated into glucose must be given consideration. The first of these reactions would be the condensation of $^{14}CO_2$ (generated from oxidation of [^{14}C-2]-acetate) with pyruvate via pyruvate carboxylase activity. Little relative incorporation of plasma $^{14}CO_2$ into glucose, however, has been shown (55). Secondly, ^{14}C-pyruvate may label acetyl CoA by way of pyruvate dehydrogenase. This pathway is expected to be of little consequence, especially in fasted animals (21). The activity of pyruvate kinase which converts ^{14}C-phosphoenolpyruvate (^{14}C-PEP) into ^{14}C-pyruvate resulting in heavy labelling of plasma lactate and alanine (55) will introduce a second flux of ^{14}C into the OAA pool and introduce error into the correction factor. In addition, Kelleher (66) suggests that the following pathways which are not accounted for may alter the correction factor and thus the rate of gluconeogenesis: *1)* the flux of 4- and 5-carbon compounds into the Krebs cycle, *2)* the reversible exchanges between OAA and fumarate and OAA and pyruvate and, *3)* the incomplete equilibrium of OAA produced in the Krebs cycle and OAA derived from pyruvate. In light of these considerations, the product of the calculated rate of gluconeogenesis and *H* is to be considered an index of gluconeogenesis (or glyconeogenesis) rather than a quantitive rate.

^{14}C-Lactate as a Gluconeogenic Tracer

Despite the fact that lactate is a major substrate for and often used to assess gluconeogenesis (and glyconeogenesis), it poses a number of problems as outlined by Radziuk (88). *1)* The true precursor for gluconeogenesis from lactate is hepatocyte lactate which may not be in equilibrium with plasma lactate. The specific activities of hepatic and plasma lactate, then, may not be in equilibrium. *2)* The lactate label is rapidly and extensively exchanged with pyruvate and alanine, the specific activities of which may not necessarily be equal to that of lactate. In addition, some carbon atoms originating in lactate reach glucose via alanine and vice versa. If two separate experiments are performed in which one uses ^{14}C-lactate as the tracer and the other uses ^{14}C-alanine, the sum total of the contributions of lactate and alanine to glucose will be overestimated as some of the carbons will be accounted for twice (54). *3)* There is metabolic exchange of ^{14}C atoms from lactate for ^{12}C atoms arriving in the OAA pool from the Krebs cycle (as discussed above). 4) With labelled lactate or alanine as tracers, more metabolites become labelled with time. An example would be the formation of ^{14}C-glycerol (from ^{14}C-glucose). Since it does not pass through the OAA pool on its way to glucose, the rate of gluconeogenesis, then, would be overestimated (54). Under general circumstances, the incorporation of ^{14}C from lactate (or alanine) into glucose as an index of gluconeogenesis (or glyconeogenesis) has some limitations. In tracking a lactate load, however, it can be very useful since the large amount of exogenous lactate will be the principal determinant of lactate fluxes.

Labelled Bicarbonate as an Alternative Tracer

Radziuk (88) has proposed $^{14}CO_2$ as an alternative to labelled lactate or alanine for the assessment of gluconeogenic fluxes. CO_2 is small, highly diffusible and equilibrates rapidly with HCO_3^- (via carbonic anhydrase) allowing for infusion of the tracer in the form of $H^{14}CO_3^-$. $^{14}CO_2$ labels the pathway by condensing with pyruvate in the pyruvate carboxylase reaction to form labelled OAA. (Gluconeogenic contributions of pyruvate-level substrates, then, are traced while those of glycerol are unaccounted for.) Because OAA is in equilibrium with fumarate due to the rapid reversibilities of the malate dehydrogenase and fumarase reactions, and owing to the fact that fumarate is a symmetrical molecule, the ^{14}C-carboxyl label will be randomized between the first and fourth carbons of OAA and not simply lost in the subsequent decarboxylation by PEPCK to form PEP. In this way, $^{14}CO_2$ labels the gluconeogenic pathway without acting as a net substrate. Because of its advantages, then, $^{14}CO_2$, in the form of $NaH^{14}CO_3$, was chosen to trace hepatic gluconeogenesis in rested and exercised rats.

If the specific activity of CO_2 equals a, then following equilibration with fumarate, the specific activity of *each* of the carboxyl carbons of OAA will be $a/2$. Therefore, the specific activity of PEP will be $a/2$. But since glucose is formed from two molecules of PEP, the specific activity of glucose will be equal to a. Thus, an index of gluconeogenesis, R_{ag}, may be calculated by

$$R_{ag} = \frac{R_a^*}{a_{CO_2}} \qquad (I.3)$$

where R_a^* equals the rate of appearance of ^{14}C-glucose and a is the specific activity of plasma CO_2. (The specific activity of plasma urea may be used as an estimate of hepatocellular CO_2 specific activity as the only precursor to ^{14}C-urea is $^{14}CO_2$ in the hepatocyte (55).)

R_{ag} is an index rather than a true rate of gluconeogenesis as it is based on the following assumptions (88, 89): *1)* There is rapid diffusion of CO_2 between plasma and hepatocyte and most of the ^{14}C from $^{14}CO_2$ is incorporated at the pyruvate carboxylase step. *2)* OAA and fumarate equilibrate rapidly to allow for label randomization. *3)* Minimal recycling of label occurs via pyruvate kinase and pyruvate dehydrogenase activities. *4)* Glycerol contributes little to glucose formation. Calculation of R_{ag} becomes difficult out of steady state (eg. exercise) as the specific activity of CO_2 may change. (This, however, would be applicable to any substrate that is produced in exercise, particulary lactate.) Correction for metabolic exchange in the OAA pool can be accounted for. The correction factor H no longer applies to total gluconeogenic flux from pyruvate-level substrates when pyruvate condenses with $^{14}CO_2$ (88). Instead, a different correction factor, J, is calculated (88) but subject to many of the assumptions as discussed for H.

Figure I.3: Tracer strategies: pathways labelled by different tracers.
Panel A: Gluconeogenic glycogen formation from systemic lactate (dark arrows) is traced by [^{14}C-U]-lactate (^{14}C-Lac) infusion into plasma. [^{3}H-6]-glucose (^{3}H-6-G) is infused to track direct glycogen synthesis from plasma ^{14}C-glucose (open arrows) which arises from hepatic gluconeogenesis from ^{14}C-lactate. $^{14}CO_2$, infused in the form of $NaH^{14}CO_3$, traces glyconeogenesis in liver only since it is not incorporated via the glyconeogenic route in the muscle.
Panel B: To label glyconeogenesis from lactate formed *locally* in the muscle (dark arrows), [^{14}C-1]-glucose (^{14}C-1-G) is used. ^{14}C-glucose in glycogen arising from direct uptake of recycled ^{14}C-glucose from plasma (formed via hepatic gluconeogenesis from ^{14}C-lactate) is accounted for by infusion of [^{3}H-3]-glucose (^{3}H-3-G) which tracks direct glycogen synthesis (open arrows).

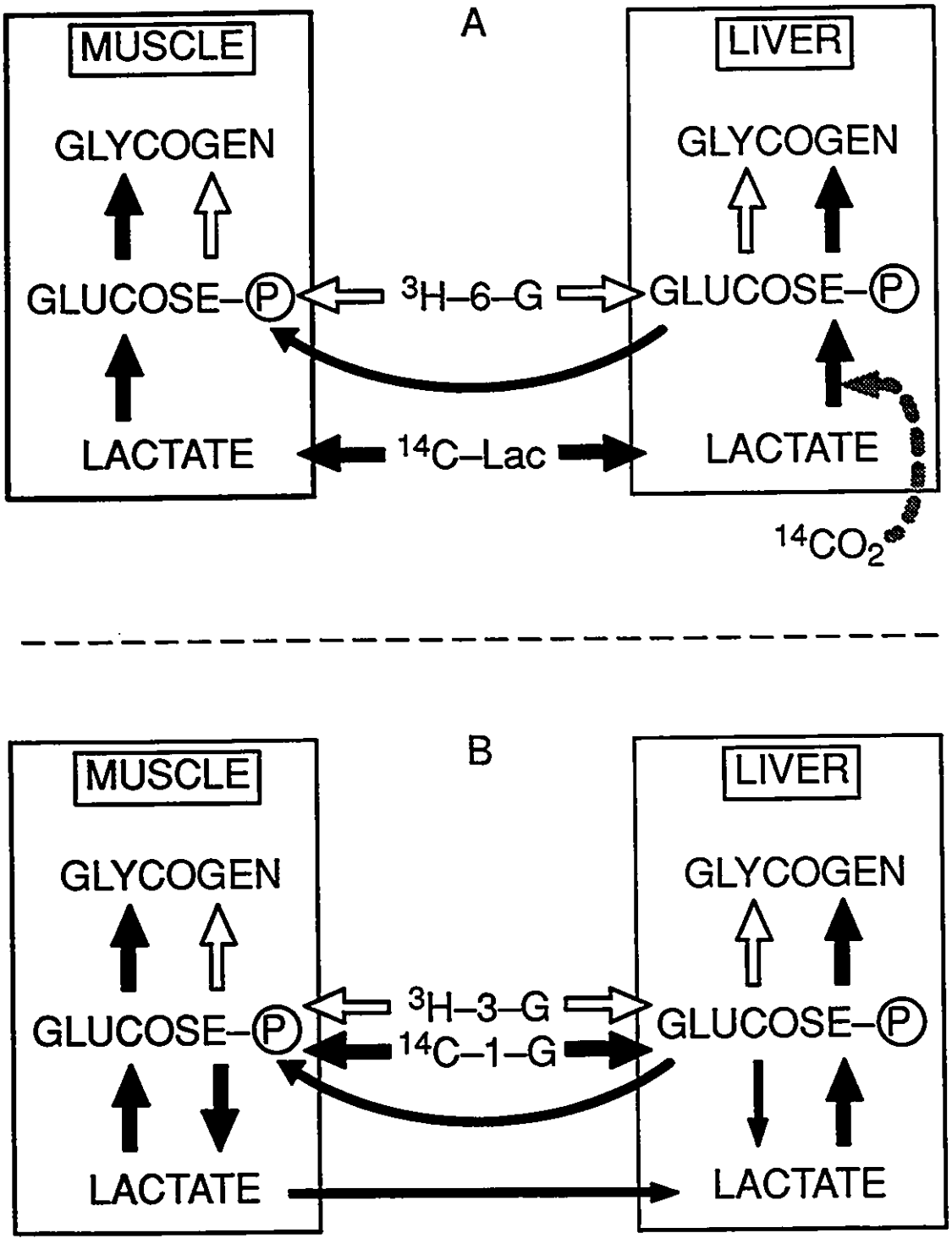

Figure I.3: Tracer strategies: pathways labelled by different tracers.

TRACER METHODS AS APPLIED TO MUSCLE GLYCOGEN

^{14}C-Lactate as a Glyconeogenic Tracer in Muscle

The tracer approach taken in address to the question as to whether there is any significant gluconeogenic synthesis of glycogen in muscle is based on the principles of that which was used previously with respect to detection of hepatic (79, 88) and muscle glyconeogenesis (62, 101). If muscle glycogen is made entirely from plasma glucose, then the ratios of $^{3}H/^{14}C$ in glycogen would be the same as those in plasma glucose following infusion of [^{3}H-6]-glucose and [^{14}C-U]-lactate (the ^{14}C-glucose in plasma and glycogen arising from hepatic glucose production from [^{14}C-U]-lactate). (Refer to Figure I.3, p. 32, Panel A). This is due to the loss of the tritium label from the sixth carbon in glucose in the gluconeogenic pathway; thus, [^{3}H-6]-glucose traces only the direct uptake of glucose into muscle glycogen. If, however, there is significant incorporation of ^{14}C into glycogen via glyconeogenesis from [^{14}C-U]-lactate, then the ratio of $^{3}H/^{14}C$ in glycogen will be significantly lower than that in plasma. Experimentally, then, the ^{14}C in glycogen not arising from ^{14}C-glucose in plasma is used as an index of muscle glyconeogenesis.

The amount of ^{14}C-glycogen that could be accounted for by the direct uptake of plasma glucose could be calculated based on the assumption that the ratio of $^{3}H/^{14}C$ in glycogen arising directly from glucose must be equal to that of plasma glucose:

$$\frac{[^{3}H]\text{-}glucose}{[^{14}C]\text{-}glucose} = \frac{[^{3}H]\text{-}glycogen}{[^{14}C]\text{-}glycogen}$$

Rearranging the equation gives

$$direct\ [^{14}C]\text{-}glycogen = \frac{tissue\ [^{3}H]\text{-}glycogen\ d.p.m.}{plasma\ \frac{[^{3}H]\text{-}glucose\ d.p.m.}{[^{14}C]\text{-}glucose\ d.p.m.}} \qquad (I.4)$$

The difference, then, between the total amount of ^{14}C-d.p.m. in glycogen and that which can be accounted for by the direct uptake of ^{14}C-glucose from plasma, would represent glyconeogenic contributions from [^{14}C-U]-lactate. As this is a relative term in ^{14}C-d.p.m., a more quantitative estimate can be calculated by dividing this difference by the average specific activity of ^{14}C-lactate in plasma:

$$index = \frac{total\ [^{14}C]\text{-}glycogen - direct\ [^{14}C]\text{-}glycogen}{SA\ plasma\ [^{14}C]\text{-}lactate} \qquad (I.5)$$

This represents an *index* of glyconeogenesis due to the number of assumptions and limitations discussed previously, especially since the specific activity of plasma lactate may not be equal to that of intramuscular lactate and will not be constant during exercise. Since a correction factor for label dilution of glyconeogenic precursors has not been estimated in muscle, particularly during or following exercise, the index is a minimal estimate of glyconeogenic activity in the muscle.

Tissue glycogen synthesized from direct uptake of plasma glucose is calculated by

$$direct\ synthesis = \frac{[^{3}H]\text{-}d.p.m.\ tissue\ glycogen}{SA\ plasma\ [^{3}H]\text{-}glucose} \qquad (I.6)$$

where *[^{3}H]-d.p.m.* are those from ^{3}H-glucose in tissue glycogen and *SA* is the average specific activity of ^{3}H-glucose in plasma (C*/C) during recovery (or during exercise

for rats sacrificed immediately postexercise).

^{14}C-Glucose Recycling as a Glyconeogenic Index

[^{14}C-U]-lactate traces glyconeogenic contributions made by plasma (systemic) lactate to glycogen synthesis and may not adequately label those of intracellular lactate. In attempt to label intramuscular lactate more effectively, then, [^{14}C-1]-glucose is infused into the rats (Chapters 3 and 4). (Refer to Figure I.3, Panel B, p. 32). Theoretically, [^{14}C-1]-glucose would be taken up by muscle and oxidized to ^{14}C-lactate which could then be synthesized into muscle glycogen via the glyconeogenic route, labelling it with ^{14}C-d.p.m. But, of course, this ^{14}C-lactate could also leave the muscle and undergo hepatic gluconeogenesis to form recycled ^{14}C-glucose. (The same cycle, [^{14}C-1]-glucose → ^{14}C-lactate → ^{14}C-glucose, could also take place within the liver, but to a lesser degree.) This recycled ^{14}C-glucose would then enter the plasma and be taken up directly into muscle glycogen, also labelling it with ^{14}C-d.p.m. Thus, in order to account for recycled ^{14}C-glycogen arising from recycled ^{14}C-glucose in plasma, [^{3}H-3]-glucose is infused to trace the direct route as the label in the three position is lost in the isomerization of dihydroxyacetone phosphate (DHAP) and glyceraldehyde phosphate in glycolysis and therefore cannot be recycled. Glucose tritiated on carbon three was preferred to that in the sixth position (above) as the recycling assay actually measures label on carbon six (94). Recycled glucose originating from [^{14}C-1]-glucose may be labelled in the 1, 2, 5, or 6 positions (95). This allows for quantitation by cleavage of the glucose molecules and label assessment

in the C-6 postions in plasma glucose and glycogen (94), thus distinguishing recycled ^{14}C-glucose from the [^{14}C-1]-glucose tracer.

Labelled Bicarbonate as a Control for Muscle Glyconeogenesis

A number of assumptions are inherent in the above measurements of glyconeogenesis. These include the linearity of glycogen formation which, in turn, allows us to average the changing plasma substrate specific activities during the glycogen synthetic period examined (i.e. exercise or recovery). Fortunately, this assumption can be tested to some extent by using $^{14}CO_2$ as a label. $^{14}CO_2$ is not incorporated into muscle glycogen via glyconeogenesis as in liver (56) due to inadequate equilibration in the fumarase reaction in muscle (14). As no label incorporation is expected in muscle in $NaH^{14}CO_3$-infused rats, any labelling of muscle glycogen apart from that arising from the uptake of plasma ^{14}C-glucose derived from hepatic gluconeogenesis would be due to deviations from the above assumptions or to the intrinsic errors in the methods used. It can thus be used as a "zero" or minimal detectable error and the basis of the statistical analysis for the above glyconeogenic tracer methods (see Chapter 2 for details).

UNIQUE APPROACH

Our experimental approach, then, to the question of muscle glyconeogenesis is unique for the following reasons:

1) Using the prolonged swimming rat model, the effects of exercise, *per se*, on

muscle glyconeogenesis can be separated from those of lactate alone, as well as taken in conjunction with influences of high substrate availability (produced by exogenous infusion).

2) The use of different labelling techniques, [^{14}C-U]-lactate and [^{14}C-1]-glucose, allows for a distinction (to some degree) between glyconeogenic contributions made by systemic and locally formed (intramuscular) lactate. The latter issue has not been specifically addressed in the literature.

3) The use of labelled bicarbonate allows for a control which tests the physiological assumptions made and which can be used as a statistical "zero" for muscle glyconeogenic activity detected by the above tracer methods. Simultaneously, it permits an estimation of hepatic gluconeogenic activity during prolonged swimming and recovery.

REFERENCES

1. Ahlborg, G., J. Bergström, L. -G. Ekelund and E. Hultman. Muscle glycogen and muscle electrolytes during prolonged physical exercise. Acta Physiol. Scand. 70: 129-142, 1967.

2. Ahlborg, G., and P. Felig. Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. J. Clin. Invest. 69: 45-54, 1982.

3. Ahlborg, G., P. Felig, L. Hagenfeldt, R. Hendler, and J. Wahren. Substrate turnover during prolonged exercise in man. J. Clin. Invest. 53: 1080-1090, 1974.

4. Ariano, M. A., R. B. Armstrong, and V. R. Edgerton. Hindlimb muscle fiber

populations of five mammals. J. Histochem. Cytochem. 21(1): 51-55, 1973.

5. Armstrong, R. B., and R. O. Phelps. Muscle fiber type composition of the rat hindlimb. Am. J. Anat. 171: 259-272, 1984.

6. Armstrong, R. B., C. W. Saubert, W. L. Sembrowich, R. E. Shepard, and P. D. Gollnick. Glycogen depletion in rat skeletal muscle fibers at different intensities and durations of exercise. Pflügers Arch. 352: 243-256, 1974.

7. Åstrand, P. -O., E. Hultman, A. Juhlin-Dannfelt, and G. Reynolds. Disposal of lactate during and after strenuous exercise in humans. J. Appl. Physiol. 61(1): 338-343, 1986.

8. Bagby, G. J., H. J. Green, S. Katsuta, and P. D. Gollnick. Glycogen depletion in exercising rats infused with glucose, lactate or pyruvate. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 45(3): 425-429, 1978.

9. Baker, N. Measurement of glucose recycling and liver glycogen synthesis in mice using doubly labelled substrates. Fed. Proc. 36: 253-258, 1977.

10. Baldwin, K. H., J. S. Reitman, R. J. Terjung, W. W. Winder, and J. O. Holloszy. Substrate depletion in different types of muscle and in liver during prolonged running. Am. J. Physiol. 255(5): 1045-1050, 1973.

11. Bendall, J. R., and A. A. Taylor. The Meyerhof quotient and the synthesis of glycogen from lactate in frog and rabbit muscle. Biochem. J. 118: 887-803, 1970.

12. Bergström, J., L. Hermansen, E. Hultman, and B. Saltin. Diet, muscle glycogen and physical performance. Acta Physiol. Scand. 71: 140-150, 1967.

13. Björkman, O., and L. S. Eriksson. Splanchnic glucose metabolism during leg exercise in 60-hour-fasted human subjects. Am. J. Physiol. 245 (Endocrinol. Metab. 8): E443-E448, 1983.

14. Bloom, B., and W. Foster. Source of phosphoenolpyruvate for hexose synthesis in liver and muscle as studied with aspartate-3-C^{14}. J. Biol. Chem. 237(9): 2744-2746, 1962.

15. Bonen, A., J. C. McDermott, and M. H. Tan. Glycogenesis and glyconeogenesis in skeletal muscle: effects of pH and hormones. Am. J. Physiol. 258 (Endocrinol. Metab. 21): E693-E700, 1990.

16. Bonen, A., G. W. Ness, A. N. Belcastro, and R. L. Kirby. Mild exercise

impedes glycogen repletion in muscle. J. Appl. Physiol. 58(2): 1622-1629, 1985.

17. Brooks, G. A. Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. Fed. Proc. 45: 2924-2929, 1986.

18. Brooks, G. A. The lactate shuttle during exercise and recovery. Med. Sci. Sports Exerc. 18(3): 360-368, 1986.

19. Brooks, G. A., and C. M. Donovan. Effect of endurance training on glucose kinetics during exercise. Am. J. Physiol. 244 (Endocrinol. Metab. 7): E505-E512, 1983.

20. Brooks, G. A., and G. A. Gaesser. End points of lactate and glucose metabolism after exhausting exercise. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(6): 1057-1069, 1980.

21. Brosnan, J. T. Pathways of carbon flux in gluconeogenesis. Fed. Proc. 41: 91-95, 1982.

22. Calles, J., J. J. Cunningham, L. Nelson, N. Brown, E. Nadel, R. S. Sherwin, and P. Felig. Glucose turnover during recovery from intensive exercise. Diabetes 32: 734-738, 1983.

23. Chasiotis, D., K. Sahlin, and E. Hultman. Regulation of glycogenolysis in human muscle at rest and during exercise. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 53(3): 708-715, 1982.

24. Cheosakul, P., L. W. Blide, M. Vanko, and A. Knudson. Conversion of lactate into glycogen in skeletal muscle of hepatectomized rats. Science 123: 375-376.

25. Conlee, R. K. Muscle glycogen and exercise endurance: a twenty-year perspective. Exerc. Sport Sci. Rev. 15: 1-28, 1987.

26. Conlee, R. K., R. C. Hickson, W. W. Winder, J. M. Hagberg, and J. O. Holloszy. Regulation of glycogen resynthesis in muscles of rats following exercise. Am. J. Physiol. 235 (Regulatory Integrative Comp. Physiol. 4): R145-R150, 1978.

27. Conlee, R. K., J. A. McLane, M. J. Rennie, W. W. Winder, and J. O. Holloszy. Reversal of phosphorylase activation in muscle despite continued contractile activity. Am. J. Physiol. 237(5): R291-R296, 1979.

28. Connett, R. J. Glyconeogenesis from lactate in frog striated muscle. Am J. Physiol. 237(5): C231-C236, 1979.

29. Constable, S. H., J. C. Young, M. Higuchi, and J. O. Holloszy. Glycogen resynthesis in leg muscles of rats during exercise. Am. J. Physiol. 247 (Regulatory Integrative Comp. Physiol. 16): R880-R883, 1984.

30. Cooper, D. M., T. J. Barstow, A. Bergner, and W. N. P. Lee. Blood glucose turnover during high- and low-intensity exercise. Am. J. Physiol. 257 (Endocrinol. Metab. 20) E:405-E412, 1989.

31. Crabtree, B., S. J. Higgins, and E. A. Newsholme. The activities of pyruvate carboxylase, phosphoenolpyruvate carboxylase and fructose diphosphatase in muscles from vertebrates and invertebrates. Biochem. J. 130: 391-396, 1972.

32. deBodo, R. C., R. Steele, N. Altszuler, A. Dunn, and J. S. Bishop. On the hormonal regulation of carbohydrate metabolism, studies with ^{14}C-glucose. Recent Prog. Horm. Res. 19: 445-482, 1963.

33. Dohm, G. L., R. T. Beeker, G. Israel, and E. B. Tapscott. Metabolic responses to exercise after fasting. J. Appl. Physiol. 61(4): 1363-1368, 1986.

34. Dohm, G. L., G. J. Kasperek, and H. A. Barakat. Time course changes in gluconeogenic enzymes activities during exercise and recovery. Am. J. Physiol. 249 (Endocrinol. Metab. 12): E6-E11, 1985.

35. Dohm, G. L., and E. A. Newsholme. Metabolic control of hepatic gluconeogenesis during exercise. Biochem. J. 212: 633-639, 1983.

36. Dohm, G. L., E. B. Tapscott, H. A. Barakat, and G. J. Kasperek. Influence of fasting on glycogen repletion in rats during exercise. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 55(3): 830-833, 1983.

37. Donovan, C. M., and G. A. Brooks. Endurance training affects lactate clearance, not lactate production. Am. J. Physiol. 244 (Endocrinol. Metab. 7): E83-E92, 1983.

38. Donovan, C. M., and K. D. Sumida. Training improves glucose homeostasis in rats during exercise via glucose production. Am. J. Physiol. 258 (Regulatory Integrative Comp. Physiol. 27): R770-R776, 1990.

39. Dunn, A., M. Chenoweth, and L. D. Schaeffer. Estimation of glucose turnover and the Cori cycle using glucose-6-*t*-^{14}C. Biochemisty 6(1): 6-11, 1967.

40. Dyson, R. D., J. M. Cardenas, and R. J. Barsotti. The reversibility of skeletal muscle pyruvate kinase and an assessment of its capacity to support glyconeognesis.

J. Biol. Chem. 250(9): 3316-3321, 1975.

41. Eggleton, M. G., and C. L. Evans. The lactic acid content of the blood after muscular contraction under experimental conditions. J. Physiol. (Lond.) 70: 269-293, 1930.

42. Essén, B., E. Jansson, J. Henriksson, A. W. Taylor, and B. Saltin. Metabolic characteristics of fiber types in human skeletal muscle. Acta Physiol. Scand. 95: 153-165, 1975.

43. Favier, R. J., H. E. Koubi, M. H. Mayet, B. Semporé, B. Simi, and R. Flandrois. Effects of gluconeogenic precursor flux alterations on glycogen resynthesis after prolonged exercise. J. Appl. Physiol. 63(5): 1733-1738, 1987.

44. Fell, R. D., J. A. McLane, W. W. Winder, and J. O. Holloszy. Preferential resynthesis of muscle glycogen in fasting rats after exhausting exercise. Am. J. Physiol. 238 (Regulatory Integrative Comp. Physiol. 7): R238-R332, 1980.

45. Foster, D. W. From glycogen to ketones - and back. Diabetes 33: 1188-1199, 1984.

46. Gaesser, G. A., and G. A. Brooks. Glycogen repletion following continous and intermittent exercise to exhaustion. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(4): 722-728, 1980.

47. Gleeson, T. Glycogen synthesis from lactate in skeletal muscle of the lizard *Dipsosaurus dorsalis*. J. Comp. Physiol. B. 156: 277-283, 1985.

48. Goebel, R., M. Berman, and D. Foster. Mathematical model for the distribution of isotopic carbon atoms through the tricarboxylic acid cycle. Fed. Proc. 41: 96-103, 1982.

49. Goodman, M. N., R. Dietrich, and P. Luu. Formation of gluconeogenic precursors in rat skeletal muscle during fasted-refed transition. Am. J. Physiol. 259 (Endocrinol. Metab. 22): E513-E516, 1990.

50. Hamilton, N. E. G. Noble, and C. D. Ianuzzo. Glycogen repletion in different skeletal muscles from diabetic rats. Am. J. Physiol. 247 (Endocrinol. Metab. 10): E740-E746, 1984.

51. Hems, D. A., P. O. Whitton, and E. A. Taylor. Glycogen synthesis in the perfused liver of the starved rat. Biochem. J. 129:529-538, 1972.

52. Hermansen, L, E. Hultman, and B. Saltin. Muscle glycogen during prolonged severe exercise. Acta Physiol. Scand. 71: 129-139, 1967.

53. Hermansen, L., and O. Vaage. Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. Am J. Physiol. 233(5): E422-E429, 1977.

54. Hetenyi, G., Jr. Correction for the metabolic exchange of ^{14}C for ^{12}C atoms in the pathway of gluconeogenesis *in vivo*. Fed. Proc. 41: 104-109, 1982.

55. Hetenyi, G., Jr., B. Lussier, C. Ferrarotto, and J. Radziuk. Calculation of the rate of gluconeogenesis from the incorporation of ^{14}C atoms from labelled bicarbonate or acetate. Can. J. Physiol. Pharmacol. 60: 1603-1609, 1982.

56. Hiatt, H. H., M. Goldstein, J. Laureau, and B. L. Horecker. The pathway of hexose synthesis from pyruvate in muscle. J. Biol. Chem. 231: 303-307, 1958.

57. Hill, A. V. Muscular activity and carbohydrate metabolism. Science Wash. DC 60: 505-514, 1924.

58. Holness, M. J., M. J. L. Schuster-Bruce, and M. C. Sugden. Skeletal-muscle glycogen synthesis during the starved-to-fed transition in the rat. Biochem. J. 254: 855-859, 1988.

59. Hultman, E. H. Carbohydrate metabolism during hard exercise and in the recovery period after exercise. Acta Physiol. Scand. 128 (Supp. 556): 75-82, 1986.

60. Hutber, C. A., and A. Bonen. Glycogenesis in muscle and liver during exercise. J. Appl. Physiol. 66(6): 2811-2817, 1989.

61. John-Alder, H. B., R. M. McAllister, and R. L. Terjung. Reduced running endurance in gluconeogenesis-inhibited rats. Am. J. Physiol. 251 (Regualtory Integrative Comp. Physiol. 20): R137-R142, 1986.

62. Johnson, J. L., and G. J. Bagby. Gluconeogenic pathway in liver and muscle glycogen synthesis after exercise. J. Appl. Physiol. 64(4): 1591-1599, 1988.

63. Karlsson, J. and B. Saltin. Diet, muscle glycogen, and endurance performance. J. Appl. Physiol. 31(2): 203-206, 1971.

64. Katz, J. Importance of sites of tracer administration and sampling in turnover studies. Fed. Proc. 41: 123-128, 1982.

65. Katz, J., and J. D. McGarry. The glucose paradox: is glucose a substrate for

65. Katz, J., and J. D. McGarry. The glucose paradox: is glucose a substrate for liver metabolism? J. Clin. Invest. 74: 1901-1909, 1984.

66. Kelleher, J. K. Gluconeogenesis from labeled carbon: estimating isotope dilution. Am. J. Physiol. 250 (Endocrinol. Metab. 13): E296-E305, 1986.

67. Kochan, R. G., D. R. Lamb, S. A. Lutz, C. V. Perrill, E. M. Reimann, and K. K. Schlender. Glycogen synthase activation in human skeletal muscle: effects of diet and exercise. Am. J. Physiol. 236(6): E660-E666, 1979.

68. Krebs, H. A., and M. Woodford. Fructose 1,6-diphosphatase in striated muscle. Biochem. J. 94: 436-445, 1965.

69. Kurland, I. J., and S. J. Pilkis. Indirect *versus* direct routes of hepatic glycogen synthesis. FASEB J. 3: 2277-2281, 1989.

70. Landau, B. R. and J. Wahren. Quantification of the pathways followed in hepatic glycogen formation from glucose. FASEB J. 2: 2368-2375, 1988.

71. Lang, C. H., G. J. Bagby, H. L. Blakesley, J. L. Johnson, and J. J. Spitzer. Plasma glucose concentration determines direct versus indirect liver glycogen synthesis. Am. J. Physiol. 251 (Endocrinol. Metab. 14): E584-E590, 1986.

72. Maehlum, S., P. Felig, and J. Wahren. Splanchnic glucose and muscle glycogen metabolism after glucose feeding during postexercise recovery. Am. J. Physiol. 235(3): E255-E260, 1978.

73. MacDonald, M., N. Neufeldt, B. N. Park, M. Berger, and N. B. Ruderman. Alanine metabolism and gluconeogenesis in the rat. Am. J. Physiol. 231(2): 619-625, 1976.

74. McLane, J. A., and J. O. Holloszy. Glycogen synthesis from lactate in the three types of skeletal muscle. J. Biol. Chem. 254: 6548-6553, 1979.

75. Mitrakou, A., R. Jones, Y. Okuda, J. Pena, N. Nurjhan, J. B. Field, and J. E. Gerich. Pathway and carbon sources for hepatic glycogen repletion in dogs. Am. J. Physiol. 260 (Endocrinol. Metab. 23): E194 E202, 1991.

76. Meyerhof, O. Uber die Energieumwandlungen in Muskel. II. Das Schicksal der Erholungsperiode des Muskels. Pflügers Arch. 182: 284-317.

77. Meyerhof, O., K. Lohman, and R. Meier. Uber die Synthes des Kohlehydrats im Muskel. Biochem. Z. 157: 459-461.

78. Newgard, C. B., S. V. Moore, D. W. Foster, and J. D. McGarry. Efficient hepatic glycogen synthesis in refeeding rats requires continued carbon flow through the gluconeogenic pathway. J. Biol. Chem. 259: 6958-6963, 1984.

79. Newgard, C. B., L. J. Hirsch, D. W. Foster, and J. D. McGarry. Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat. J. Biol. Chem. 258: 8046-8052, 1983.

80. Opie, L. H., and E. A. Newsholme. The activities of fructose 1,6-diphosphatase, phosphofructokinase and phosphoenolpyruvate carboxykinase in white muscle and red muscle. Biochem. J. 103: 391-399, 1967.

81. Pagliassotti, M. J., C. M. Donovan. Glycogenesis from lactate in rabbit skeletal muscle fiber types. Am. J. Physiol. 258 (Regulatory Integrative Comp. Physiol. 27): R903-R911, 1990.

82. Pernow, B., and B. Saltin. Availability of substrates and capacity for prolonged heavy exercise in man. J. Appl. Physiol. 31(3): 416-422, 1971.

83. Peter, B. P., R. J. Barnard, V. R. Edgerton, C. A. Gillespie, and K. E. Stemple. Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. Biochem. 11: 2627-2633, 1972.

84. Peters Futre, E. M., T. D. Noakes, P. I. Raine, and S. E. Terblanche. Muscle glycogen repletion during active postexercise recovery. Am. J. Physiol. 253 (Endocrinol. Metab. 16): E305-E311, 1987.

85. Prior, R. L. Gluconeogenesis in the ruminant fetus: evaluation of conflicting evidence from radiotracer and other experimental techniques. Fed. Proc. 41: 117-122, 1982.

86. Radziuk, J. Glucose and glycogen metabolism following glucose ingestion in man: a turnover approach. In Carbohydrate Metabolism (C. Cobelli and R. N. Bergman, eds.), John Wiley & Sons Ltd., Chichester, pp. 239-266, 1981.

87. Radziuk, J. Hepatic glycogen formation by direct uptake of glucose following oral glucose loading in man. Can. J. Physiol. Pharmacol. 57: 1190-1199, 1979.

88. Radziuk, J. Sources of carbon in hepatic glycogen synthesis during absorption of an oral glucose load in humans. Fed. Proc. 41:110-116, 1982.

89. Radziuk, J. Tracer methods and the metabolic disposal of a carbohydrate load in man. Diabetes/Metabolism Reviews 3(1): 231-267, 1987.

90. Radziuk, J. Hepatic glycogen in humans. I. Direct formation after oral and intravenous glucose or after a 24-h fast. Am. J. Physiol. 257 (Endocrinol. Metab. 20): E145-E157, 1989.

91. Radziuk, J. Hepatic glycogen in humans. II. Gluconeogenetic formation after oral and intravenous glucose. Am. J. Physiol. 257 (Endocrinol. Metab. 20): E158-E169, 1989.

92. Radziuk, J, T. J. McDonald, D. Rubenstein and J. Dupre. Initial splanchnic extraction of ingested glucose in normal man. Metabolism 27: 657-669, 1978.

93. Radziuk, J., K. H. Norwich, and M. Vranic. Experimental validation of measurements of glucose turnover in nonsteady state. Am. J. Physiol. 234(1): E84-E93, 1978.

94. Reeves, R. E. The estimation of primary carbinol groups in carbohydrates. J. Amer. Chem. Soc. 63: 1476-1477, 1941.

95. Reichard, G. A., Jr., N. F. Mourey, Jr., N. J. Hochella, A. L. Patterson, and S. Weinhouse. Quantitative estimation of the Cori cyle in the human. J. Biol. Chem. 238(2): 495-501, 1963.

96. Richter, E. A. L. P. Garetto, M. N. Goodman, and N. B. Ruderman. Muscle glucose metabolism following exercise in the rat: increased sensitivity to insulin. J. Clin. Invest. 69: 785-793, 1982.

97. Sacks, J., and W. C. Sacks. Carbohydrate changes during recovery from muscular contraction. Am. J. Physiol. 112: 565-572, 1935.

98. Saltin, B. Muscle fibre recruitment and metabolism in prolonged exhaustive dynamic exercise. In Human Muscle Fatigue: Physiological Mechanisms, Pitman Medical, London, pp. 41-58, 1981.

99. Scofield, R. F., L. Kosugi, W. C. Shumann, K. Kumaran, and B. R. Landau. Quantitative estimation of the pathways followed in the conversion to glycogen of glucose administered to the fasted rat. J. Biol. Chem. 260: 8777-8782, 1985.

100. Shikama, H. and M. Ui. Glucose load diverts hepatic gluconeogenic product from glucose to glycogen *in vivo*. Am. J. Physiol. 235(4): E354-E360, 1978.

101. Shiota, M., S. Golden, and J. Katz. Lactate metabolism in the perfused rat hindlimb. Biochem. J. 222: 281-292, 1984.

102. Sonne, B., and H. Galbo. Carbohydrate metabolism during and after exercise in rats: studies with radioglucose. J. Appl. Physiol. 59(5): 1627-1639, 1985.

103. Sonne, B., and H. Galbo. Carbohydrate metabolism in fructose-fed and food-restricted running rats. J. Appl. Physiol. 61(4): 1457-1466, 1986.

104. Sonne, B., K. J. Mikines, and H. Galbo. Glucose turnover in 48-hour-fasted running rats. Am. J. Physiol. 252 (Regulatory Integrative Com. Physiol. 21): R587-R593, 1987.

105. Spriet, L. L., S. J. Peters, G. J. F. Heigenhauser, and N. L. Jones. Rat skeletal muscle triacylglycerol utilization during exhaustive swimming. Can. J. Physiol. Pharmacol. 63: 614-618, 1985.

106. Steele, R. Influences of glucose loading and of injected insulin on hepatic glucose output. Ann. N.Y. Acad. Sci. 82: 420-430, 1959.

107. Stevenson, R. W., D. R. Mitchell, G. K. Hendrick, R. Rainey, A. D. Cherrington, and R. T. Frizzell. Lactate as substrate for glycogen resynthesis after exercise. J. Appl. Physiol. 62(6): 2237-2240, 1987.

108. Talmadge, R. J., J. I. Scheide, and H. Silverman. Glycogen synthesis from lactate in chronically active muscle. J. Appl. Physiol. 66(5):2231-2238, 1989.

109. Talmadge, R. J., and H. Silverman. Glyconeogenic and glycogenic enzymes in chronically active and normal skeletal muscle. J. Appl. Physiol. 71(1): 182-191, 1991.

110. Tan, M. H., A. Bonen, W. Watson-Wright, D. Hood, M. Sopper, D. Currie, A. N. Belcastro, and G. Pierce. Muscle glycogen repletion after exercise in trained normal and diabetic rats. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 57(5): 1404-1408, 1984.

111. Terblanche, S. E., R. D. Fell, A. C. Juhlin-Dannfelt, B. W. Craig, and J. O. Holloszy. Effects of glycerol feeding before and after exhausting exercise in rats. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 50(1): 94-101, 1981.

112. Terjung, R. L., K. M. Baldwin, P. A. Molé, G. H. Klinkerfuss, and J. O. Holloszy. Effect of running to exhaustion on skeletal muscle mitochondria: a biochemical study. Am. J. Physiol. 223(3): 549-554, 1974.

113. Terjung, R. L., K. M. Baldwin, W. W. Winder, and J. O. Holloszy. Glycogen repletion in different types of muscle and in liver after exhausting exercise.

Am. J. Physiol. 226(6): 1387-1391, 1974.

114. Utter, M. F. The role of CO_2 fixation in carbohydrate utilization and synthesis. Ann. N.Y. Acad. Sci. 72: 451-461, 1959.

115. Villee, C. A., V. K. White, and A. B. Hastings. Metabolism of C^{14}-labeled glucose and pyruvate by rat diaphragm muscle *in vitro*. J. Biol. Chem. 195: 287-297, 1952.

116. Vissing, J., B. Sonne, and H. Galbo. Role of metabolic feedback regulation in glucose production of running rats. Am. J. Physiol. 255 (Regulatory Integrative Comp. Physiol. 24): R400-R406, 1988.

117. Vissing, J., J. L. Wallace, and H. Galbo. Effect of liver glycogen content on glucose production in running rats. J. Appl. Physiol. 66(1): 318-322, 1989.

118. Wasserman, D. H., and A. D. Cherrington. Hepatic fuel metabolism during muscular work: role and regulation. Am. J. Physiol. 260: E811-E824, 1991.

119. Weinman, E. O., E. H. Strisower, and I. L. Chaikoff. Conversion of fatty acids to carbohydrate: application of isotopes to this problem and role of the Krebs cycle as a synthetic pathway. Physiol. Rev. 37: 252-272, 1957.

120. Winder, W. W. Control of hepatic glucose production during exercise. Med. Sci. Sports Exerc. 17(1): 2-5, 1985.

121. Winder, W. W., M. A. Beattie, and R. T. Holman. Endurance training attenuates stress hormone responses to exercise in fasted rats. Am. J. Physiol. 243 (Regulatory Integrative Comp. Physiol. 12): R179-R184, 1982.

122. Winder, W. W., R. T. Holman, and S. J. Garhart. Effect of endurance training on liver cAMP response to prolonged submaximal exercise. Am. J. Physiol. 240 (Regulatory Integrative Comp. Physiol. 9): R330-R334, 1981.

123. Winder, W. W., M. L. Terry, and V. M. Mitchell. Role of plasma epinephrine in fasted exercising rats. Am. J. Physiol. 248 (Regulatory Integrative Comp. Physiol. 17): R302-R307, 1985.

124. Wolfe, R. R. Tracers In Metabolic Research: Radioisotope and Stable Isotope / Mass Spectrophotometry Methods. Alan R. Liss, Inc., New York, 287 pp., 1984.

CHAPTER 1: GLUCOSE DYNAMICS AND GLUCONEOGENESIS DURING AND FOLLOWING PROLONGED SWIMMING IN RATS

INTRODUCTION

The effects of moderate to intense exercise on glucose turnover have been well documented. In exercising rats, plasma glucose concentrations have been shown to increase (29, 31, 35), decrease (3, 9, 12) or be maintained (3, 39) as dictated by changes in rates of glucose production (R_a) and clearance (MCR) depending on the duration and intensity of the exercise and on the preexercise nutritional and training status. The emphasis has been almost exclusively on one type of exercise, treadmill running, at moderate to high intensities for shorter durations (less than 60 minutes) accompanied by limited recovery periods. In such exercise models, glycogen is expected to be the primary source of fuel, particularly during the early stages, with gluconeogenesis increasing in importance as exercise duration progresses (36). Less information is available, however, on glucose dynamics during and following lower intensity prolonged forms of exercise in rats.

It is expected that prolonged exercise at submaximal intensities will demonstrate different patterns of substrate utilization with resulting differences in glucose dynamics, particularly in a fasted state in which hepatic glycogen is depleted and would be deemphasized as a fuel source. In untrained rats, prolonged submaximal exercise (eg. prolonged swimming) is characterized by minimal lactate accumulation (10) and three-fold increases in plasma free fatty acids (FFA) (32). In addition, treadmill running at low intensities elicits respiratory quotient values

CO_2 produced/O_2 consumed) (eg. 0.86) significantly lower than those of rats running at higher intensities (eg. 0.93) (3), again indicative of a greater dependence on fat rather than carbohydrate oxidation. Since prolonged exercise at a lower intensity is a more common mode of exercise and may be particularly applicable in patients with diabetes, the definition of glucose fluxes during and after this type of exercise need to be examined.

Reduced running capability and hypoglycemia have been recently demonstrated in rats by pharmacological inhibition of gluconeogenesis (21), illustrating the importance of gluconeogenesis in maintaining glucose homeostasis and work performance. However, the exercise studies to date have only been able to estimate indirectly the contributions of gluconeogenesis to glucose production during and following exercise by the differences between R_a and approximated hepatic glycogen breakdown (eg. ref. 35) or by using ^{14}C-glucose recycling as an index (9). We present a more direct assessment of the index of gluconeogenesis using tracer $^{14}CO_2$ to label the contributions of pyruvate-level substrates to glucose production as has been described previously (24). This approach also allows for the assessment of CO_2 production so that insight may be gained into substrate oxidation in these studies.

The intention, then, is to present a metabolic picture of prolonged swimming and recovery in untrained rats in terms of its effects on glucose turnover and the contributions of gluconeogenesis to glucose production.

METHODS

Male Sprague-Dawley rats (Ottawa Civic Hospital, Ottawa, Ontario) were used in this study. The animals were housed in a 12:12 hour light/dark cycle environment with free access to Purina rat chow and water. Rats weighing approximately 180 grams were anesthetized with ketamine (120 mg/kg) and xylazine (8.3 mg/kg). Catheters made of silastic medical grade tubing connected to polyethylene tubing via blunted needles were implanted in the superior vena cava via the right jugular vein and in the aortic arch via the left carotid artery to allow for intravenous infusions and arterial blood sampling, respectively. The animals were allowed to recover from surgery for four days in individual cages provided with food and water. During the final two days of recovery, the rats were swum for 30 minutes in a rectangular tank (55.5 cm width, 1.4 m length, 42 cm depth) in order to accustom them to the water (35°C). Prior to the day of a study, all rats were fasted for 12 hours overnight in order to minimize glucose absorption from the gut. Fasting was initiated after a one hour feeding period during the dark cycle.

EXPERIMENTAL PROTOCOL

The fasted rats were weighed and strapped into harnesses connected to swivel and tether systems to accomodate the lines extending from the indwelling catheters. A primed constant intravenous infusion of D-[^{3}H-6]-glucose (HPLC purified using Bio-Rad HPX-87P column) and $NaH^{14}CO_3$ in 0.9% NaCl continued for 6 or 9 hours using a Harvard compact infusion pump (Model 975). The priming dose for

D-[^{3}H-6]-glucose was approximately 4.5 μCi which was followed by a constant infusion of approximately 0.04 μCi·min^{-1}; the priming dose for $NaH^{14}CO_3$ was approximately 36 μCi followed by an infusion rate of approximately 0.3 μCi·min^{-1}. Saline was simultaneously infused at a rate of 0.055 ml·min^{-1} throughout the study. A resting basal period of 2 hours was allotted in order to establish a steady state condition afterwhich the swimmer would swim for 4 hours in water maintained at 35°C, while the control rested in its cage. One group of swimming rats (n = 7), along with its rested controls (n = 7), was sacrificed immediately following the 4 hour exercise period to assess hepatic glycogen content. The remaining swimmers (n = 7) were taken out of the water, dried off and allowed to recover for 3 hours under heat lamps along with their nonexercised controls (n = 6). During a study, arterial blood samples (1 ml) were taken at 90, 120, 180, 240, 300, 360, 420, 480 and 540 minutes and mixed with two drops of heparin (1000 U/ml). The samples were spun in a microcentrifuge (Jouan M14.11) at 1000 r.p.m. for 2 minutes for separation of blood elements and plasma. Plasma was kept for metabolite analyses while the red blood cells were resuspended in saline and reinjected into the experimental animals. Following the final blood sample, the rats were sacrificed with an intravenous injection of 65 mg of sodium pentabartital. Abdominal cavities were opened in the rats and their livers clamped frozen with aluminium tongs precooled in liquid nitrogen. Tissues were kept at -85°C pending analysis of total hepatic glycogen content.

CHEMICAL ANALYSIS

Preparation of tracer standards. In order to measure the total amount of disintegrations per minute (d.p.m.) of labelled glucose and bicarbonate infused and the recoveries of the labelled metabolites through the assays (to follow), standards of the tracer infusate were made. The infusate (1 ml) was diluted 1:100 in distilled water with 100 mg carriers of both D-glucose and sodium bicarbonate.

Deproteinization. Plasma proteins were eliminated so as to avoid any interferences they might cause in the chemical analyses of the metabolites, especially in the separation of compounds in the anionic columns. Plasma (and infusion standards) (200 μl) were thus deproteinized by precipitation with 0.4 ml 0.3 N $Ba(OH)_2$ and subsequent neutralization with 0.4 ml of matched 5% w/v $ZnSO_4$ (28). Distilled water was added (10 ml), the solution vortexed and spun in a Beckman GPR Centrifuge for 20 minutes at 2000 r.p.m. The supernatant was decanted and kept for radiolabelled glucose assessment.

Plasma glucose. Glucose label (^3H and ^{14}C) was separated from labelled organic acids and amphoteric compounds in the deproteinized plasma using Dowex anionic resin columns (1 X 8 and 50W X 8, 200-400 mesh) essentially as described previously (20). The first eluate (containing glucose and other neutral compounds) was collected. An aliquot (2 ml) was dried down under vacuum (to eliminate tritiated water), reconstituted in 0.6 ml of distilled water and counted on a Packard 2200CA

Tri-Carb Liquid Scintillation Analyzer with either 15 ml of Universol or Formula 989 as the scintillation cocktail to determine both tritiated and ^{14}C counts. Recovery of the glucose label from the columns was determined by running the deproteinized standards through the columns in parallel with the plasma samples and comparing the resulting tritiated counts to those obtained from counting 1 ml of the infusate standard directly.

In order to separate glucose from the other neutral compounds in the column eluate fraction and to quantitate radiolabelled glucose, 2 ml of the column eluate containing glucose was freeze-dried in carrier glucose (solution containing 60 mg glucose) for the formation of potassium gluconate as described by Jones and Stoodley (22). The dried sample was dissolved in anhydrous methanol in a boiling water bath. (Anhydrous solutions were used as the product is soluble in water.) A solution containing 170 mg iodine in anhydrous methanol was added. Potassium hydroxide (4% in anhydrous methanol) was added dropwise, with vortexing, over a period of 40 minutes in a bath maintained at 40°C. The solution was filtered under vacuum and the precipitate was collected as a wafer, air dried, weighed, dissolved in distilled water (1 ml) and counted in scintillation fluid. Standards of ^{3}H- and ^{14}C-glucose were used to track recovery through the potassium gluconate assay.

^{3}H- and ^{14}C-glucose d.p.m./ml plasma were calculated as follows:

$$\frac{\dfrac{d.p.m.\ (^3H,^{14}C)\ x\ T.W.}{A.W.}\ x\ dilution}{recovery} = d.p.m.\ [^3H]-,[^{14}C]-glucose/ml \quad (1.1)$$

$$recovery = \frac{wafer\ (^3H)\ d.p.m./ml\ plasma}{column\ eluate\ (^3H)\ d.p.m./ml\ plasma} \quad (1.2)$$

where *T.W.* is the theoretical weight of the potassium gluconate wafer (78 mg) and *A.W.* is the actual wafer weight. The dilution factor (*dilution*) is equal to the total volume of the deproteinized sample (11 ml) divided by the product of the amount of plasma originally deproteinized (0.2 ml) and the size of the aliquot of the column eluate used in the assay (2 ml). Recovery of the label through the assay was determined using the tritiated counts of the column eluate fraction and those obtained in the gluconate assay.

Total plasma glucose was measured directly using a Beckman Glucose Analyzer 2 or Yellow Springs Instruments 2300 Stat Glucose/L-Lactate Analyzer.

Plasma lactate. Total plasma lactate was measured using the Glucose/Lactate Analyzer directly or using a microfluorometric assay. The microfluorometric assay used for lactate determination was a modified version of that previously described (23) based on the activity of lactate dehydrogenase to react lactate with NAD to form pyruvate and NADH. The increase in NADH, measured by the increase in fluorescence, is directly proportional to the amount of lactate present in the sample.

The buffer was prepared by dissolving glycine (0.17 M), semi-carbizide HCl (0.75 M) and NaOH (0.05 M) in water and adjusting the pH to 10.5. The solution was made up to volume and filtered. To 100 ml of this glycine buffer, 80 mg of NAD was added. NAD-glycine was added to 0.5 ml of deproteinized plasma. The initial relative intensity readings on the Miles Ames Fluro-Colorimeter, with excitation and emission filters set at 360 nm and 460 nm, respectively, were taken. 200 μl of lactate dehydrogenase (50 mg/10 ml suspension, diluted 1:3) was added and the final readings were taken after exactly 2 hours incubation. A deproteinized metabolite control (containing 2.23 mM of lactate) as well as duplicate standard curves (absorbance vs. nmol lactate) were run through the assay.

Plasma CO_2. $^{14}CO_2$ was determined using modifications of a method described previously (19). Plasma (100 μl) was pipetted directly into scintillation vials which were then sealed with rubber caps containing center wells. Fluted filter paper soaked in hyamine hydroxide (0.2 ml) in the center wells traps the $^{14}CO_2$ released after injection of 0.5 ml 1 N H_3PO_4 through the rubber cap. After overnight refrigeration, the center wells were cut into clean scintillation vials; the $^{14}CO_2$ was dissolved in 0.8 ml distilled water and was counted in 15 ml of Universol or Formula 989. Recovery of $^{14}CO_2$ was determined by running 100 μl of infusate standard through the assay in parallel with the samples and comparing the ^{14}C counts to those obtained by counting 100 μl of standard infusate (with 0.2 ml of hyamine hydroxide and 0.8 ml of distilled water) directly.

Total plasma CO_2 was determined by spectrophotometric analysis using a standard Diagnostic Kit for CO_2. At pH 8.0 (Tris-buffered), CO_2 in the plasma sample condenses with PEP (1.79 mM) catalyzed by PEPCK (212 U/L) forming OAA; malate dehydrogenase (1250 U/L) then catalyzes the reduction of OAA to malate using NADH (0.4 mM), thereby forming NAD. The decline in absorbance at 340 nm measured on a spectrophotometer (Bausch & Lomb Spectronic 1001) is thus proportional to the original amount of CO_2 in the plasma. A standard curve (absorbance vs. mmol CO_2) was plotted.

Liver glycogen. Glycogen was isolated in liver samples by the method described by Hassid and Abraham (15). Frozen tissue samples of liver (in duplicate) were weighed and digested in 6.0 ml of boiling 30% KOH for 30 minutes to break up the cells. Overnight refrigeration in 1.2 volumes of 90% ethanol precipitated the glycogen. Samples were then centrifuged for 20 minutes at 2000 r.p.m. and the pellets washed with 60% ethanol to remove any remaining free glucose or other impurities and centrifuged again. After the supernatants were decanted and the pellets allowed to drain, excess alcohol remaining was dispelled by heating in a boiling water bath. Tissue glycogen was then hydrolyzed in 2.0 ml of boiling 1 N HCl for 2 hours and the cooled samples were made up to 2.0 ml with distilled water. Total glycogen, now in the form of glucose subunits, was quantitated using a spectrophotometric coupled hexokinase - glucose-phosphate dehydrogenase assay system (Glucose SR Kit). Using ATP (6 mM) and Mg^{2+} (5 mM), hexokinase (4000 U/L) catalyzes the phosphorylation

of glucose to glucose-6-phosphate which is then oxidized by glucose-6-phosphate dehydrogenase, reducing NAD to NADH in the process. The increase in absorbance at 340 nm, then, is directly proportional to the amount of glucose units in the sample. The standard curve was derived by the dilutions of a standard glucose solution in 1 N HCl.

CALCULATIONS

In spite of the sparseness of the data points, the gradual changes induced by this type of exercise allowed interpolation between the points (26) and the utilization of a simple one-compartment method for calculating the rates of appearance (R_a) and metabolic clearance (MCR) of glucose. The forms of the equations used are those of de Bodo et al. (7)

$$MCR = \frac{1}{C^*}\left[p \cdot V \cdot \frac{dC^*}{dt} - R_a^*\right] \qquad (1.3)$$

$$R_a = \frac{R_a^*}{a} - \left[\frac{p \cdot V \cdot C}{a}\frac{da}{dt}\right] \qquad (1.4)$$

where C^* is the plasma concentration of D-[^{3}H-6]-glucose, C is total plasma glucose concentration, p is pool fraction of glucose (p = 0.65), V is its volume of distribution in the pool (V = 20% of body weight), R_a^* is the rate of infusion of D-[^{3}H-6]-glucose and a is the specific activity of plasma glucose (C^*/C). Identical formulae were used for CO_2 turnover with C, C^* and a, the plasma concentration of CO_2 and tracer and

its specific activity, respectively.

Since, during exercise, the specific activity of CO_2 is not constant, the rate of incorporation of ^{14}C from CO_2 into glucose cannot be accurately calculated. An estimate or index of gluconeogenesis (R_{ag}) is therefore used here, defined by the formula

$$R_{ag} = \frac{R_a^*}{a_{CO_2}} \qquad (1.5)$$

where R_a^* is the rate of appearance ^{14}C-glucose in plasma and a_{CO2} is the mean plasma specific activity of $^{14}CO_2$ for each period (basal, exercise and recovery).

Statistical analyses on plasma data were done using Dunn's procedure for *a priori* planned multiple comparisons. Comparisons of total liver glycogen were done using analysis of variance with a completely randomized factorial design and an *a posteriori* comparison by Scheffe's procedure (using the statistical package, SAS). The level of significance for each test was set at $P < 0.05$.

RESULTS

The catheterized rats had completely recovered from their surgery after four days and had surpassed their presurgical weights. Following the overnight fast, the rats lost an average of 15 g giving them final preexperiment weights of 185 $\pm$ 3 g for rested controls (n = 13) and 184 $\pm$ 4 g for swimmers (n = 14).

Figure 1.1: Plasma glucose, lactate and CO_2 concentrations during rest, exercise and recovery in rats.
Plasma glucose (upper curve), lactate (middle curve) and CO_2 concentrations (lower curve) were determined in rats during two-hour basal (0-120 minutes), four-hour exercise (or rest) (120-360 minutes) and three-hour recovery (360-540 minutes) periods. Rested rats are represented by the dashed lines, exercised animals by the solid lines. Data are expressed as means $\pm$ SEM. *significantly different from rest ($P < 0.05$). $n = 7$ for exercised rats and $n = 6$ for rested animals.

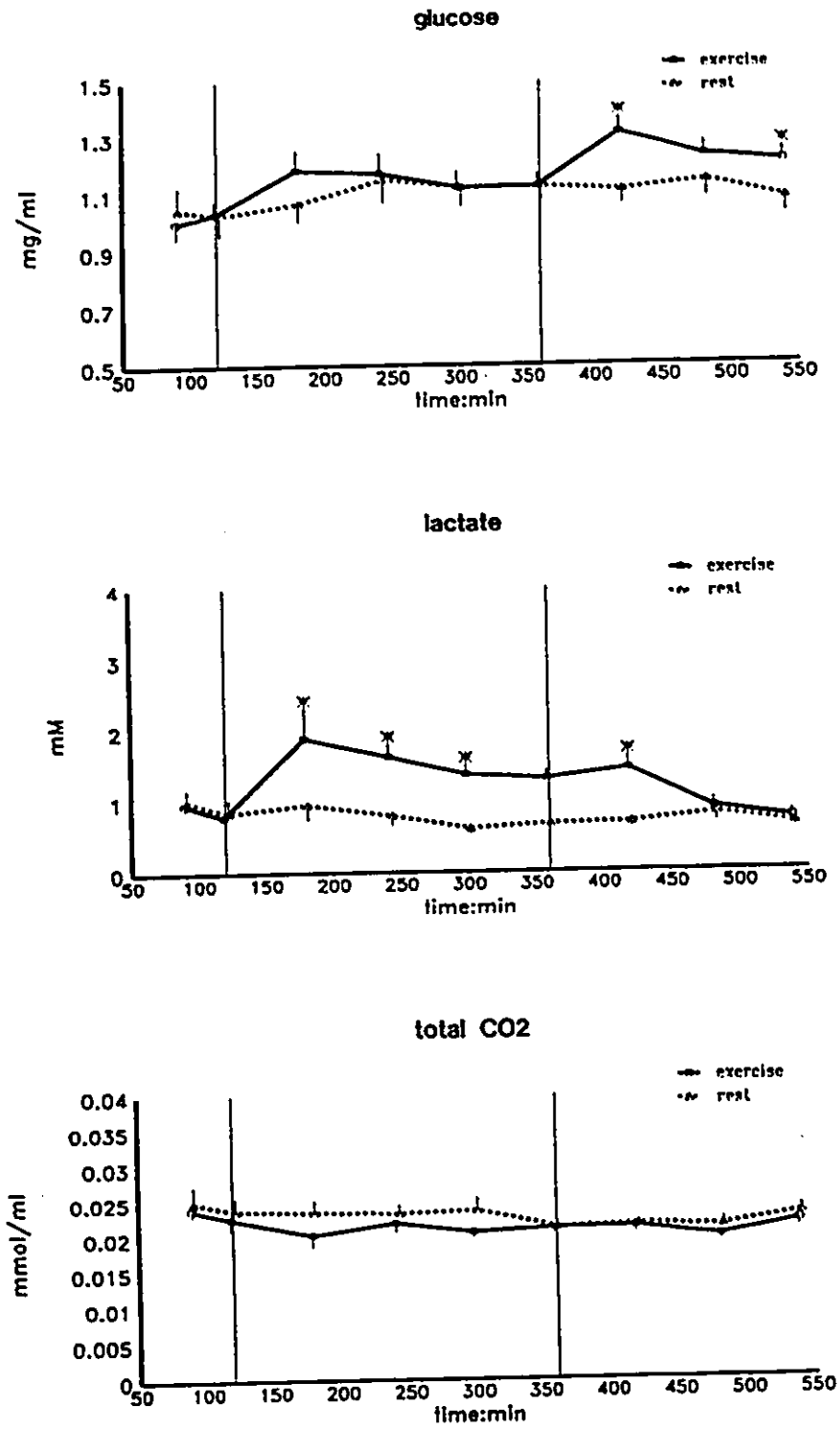

Figure 1.1: Plasma glucose, lactate and CO_2 concentrations during rest, exercise and recovery in rats.

Plasma glucose (Figure 1.1). The fasting preexercise plasma glucose levels were not different between control and exercise groups (102.5 $\pm$ 7.4 mg/dl vs. 102.9 $\pm$ 4.8 mg/dl, respectively). Despite the initial rise to 118.3 $\pm$ 6.9 mg/dl in the swimming rats during the first hour of exercise, plasma glucose concentrations in the exercising group were not significantly different from resting controls throughout the four-hour exercise period; however, glucose in the exercised rats increased during the postexercise recovery period, reaching a level significantly elevated above that of controls (131.0 $\pm$ 5.8 mg/dl vs. 110.8 $\pm$ 5.1 mg/dl, $P < 0.05$) one hour postexercise and remained significantly higher than control glucose levels after three hours of recovery.

Plasma lactate (Figure 1.1). There were no significant changes in the controls over the experimental periods; however, the swim induced only a doubling of the plasma lactate concentrations after the first hour of the exercise period (from 0.78 $\pm$ 0.05 to 1.88 $\pm$ 0.45 mM). Lactate concentrations declined gradually thereafter but remained significantly elevated as compared with rested controls for the next two hours of swimming and up to one hour postexercise before finally achieving resting levels two hours into recovery.

Plasma CO_2 (Figure 1.1). There were no significant changes in either the control or exercise group during the nine hours of the study.

Figure 1.2: Rates of glucose production and clearance during rest, exercise and recovery in rats.
The rates of glucose production (rate app, lower curve) and clearance (mcr, upper curve) were determined during two-hour basal (0-120 minutes), four-hour exercise (or rest) (120-360 minutes) and three-hour recovery (360-540 minutes) periods by the constant infusion of [^{3}H-6]-glucose into rats. Rested rats are represented by the dashed lines, exercised animals by the solid lines. Data are expressed as means $\pm$ SEM. *significantly different from rest ($P < 0.05$). $n = 7$ for exercised rats and $n = 6$ for rested animals.

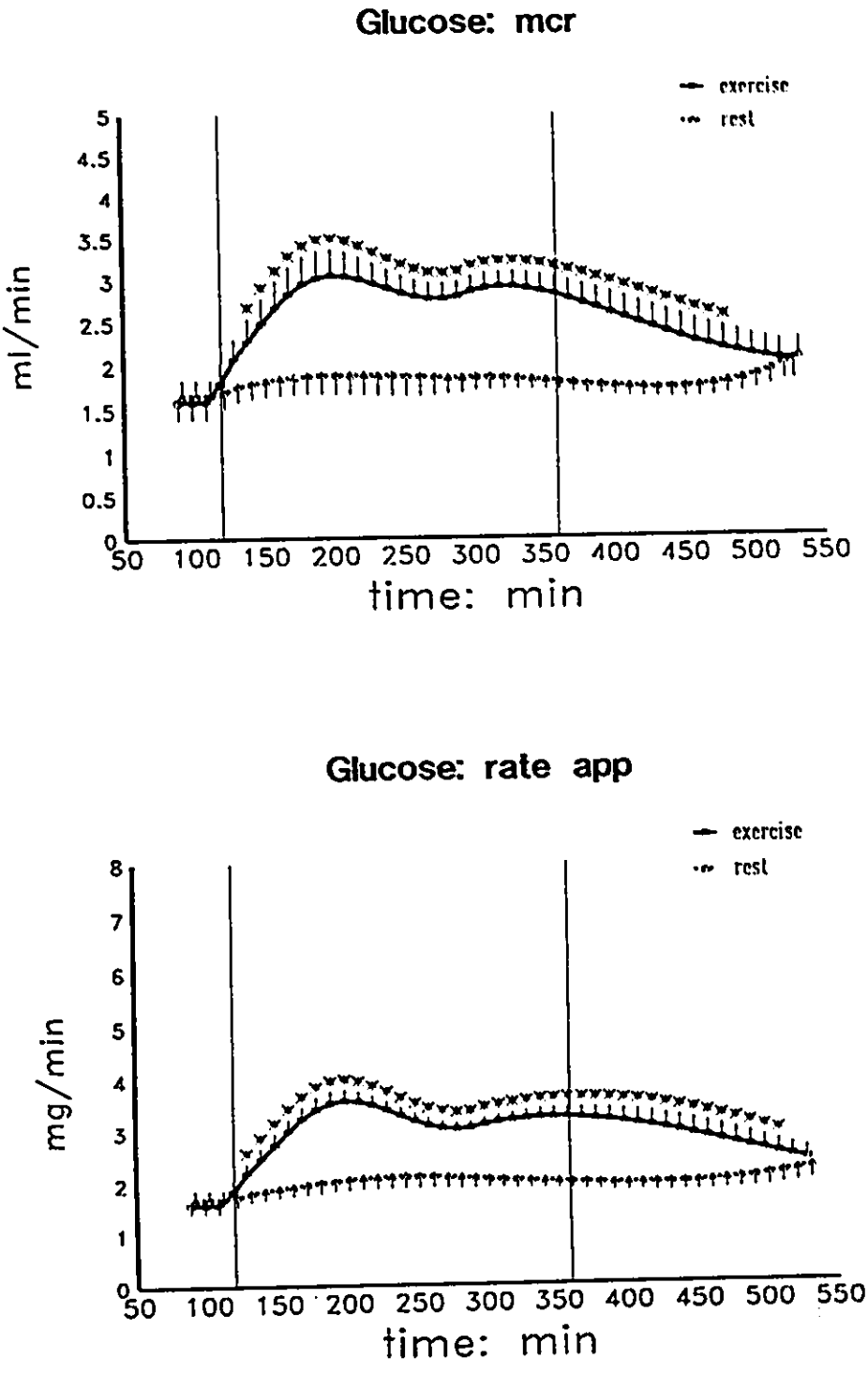

Figure 1.2: Rates of glucose production and clearance during rest, exercise and recovery in rats.

Figure 1.3: Indices of gluconeogenesis in rested and exercised rats.
Indices of gluconeogenesis were determined during two-hour basal (basal) (0-120 minutes), four-hour exercise (exer) or rest (rest) (120-360 minutes) and three-hour recovery (recov) (360-540 minutes) periods by the constant infusion of $NaH^{14}CO_3$ into rats. The mean plasma specific activity of CO_2 during each period was used to calculate the respective indices. Data are expressed as means $\pm$ SEM. *significantly different from rest ($P < 0.05$). $n = 7$ for exercised rats and $n = 6$ for rested animals.

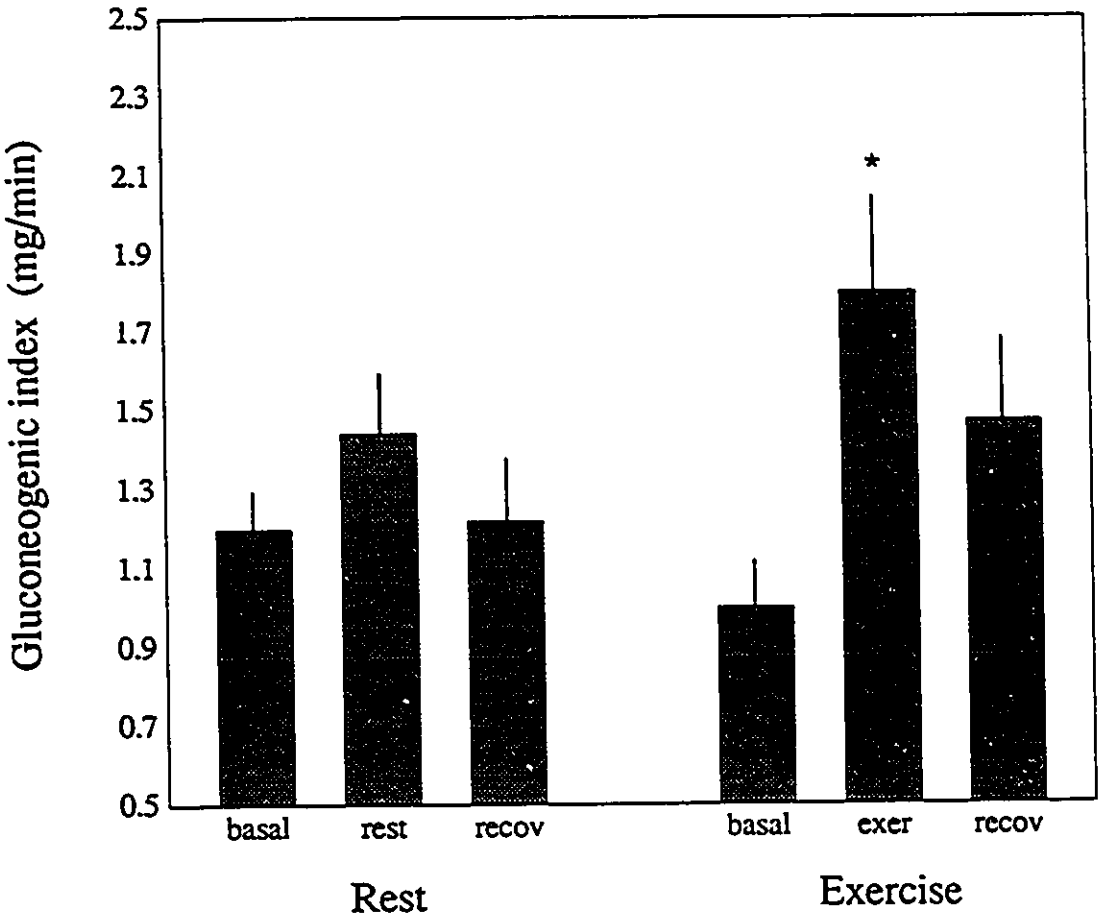

Figure 1.3: Indices of gluconeogenesis in rested and exercised rats.

Metabolic Clearance and Production of Glucose (Figure 1.2). The maintenance of the plasma glucose levels during exercise is reflected in the matched increases in MCR and R_a of glucose during this period. While there were no significant changes in glucose fluxes in control rats, R_a in the exercising group doubled from its basal rate of 1.58 $\pm$ 0.17 mg/min to 3.58 $\pm$ 0.21 mg/min after 80 minutes of swimming and remained elevated for the subsequent three hours of exercise. There was a simultaneous doubling of glucose clearance during the first hour and twenty minutes of swimming (1.58 $\pm$ 0.19 ml/min to 3.05 $\pm$ 0.32 ml/min). Glucose clearance remained significantly elevated throughout the exercise period. Both glucose R_a and MCR, however, decreased somewhat after the initial peak at about 90 minutes (after the initiation of exercise) and returned to basal levels in the exercised rats after three hours of recovery. The rate of decline in R_a lagged behind that of MCR accounting for the elevated postexercise glucose levels (Figure 1.1).

Index of gluconeogenesis (Figure 1.3). Swimming induced an 80% increase in the index of the rate of gluconeogenesis. From a resting basal rate of 1.00 $\pm$ 0.13 mg/min, the calculated index of gluconeogenesis (from the mean $^{14}CO_2$ specific activity during exercise) rose to 1.81 $\pm$ 0.23 mg/min during the exercise period ($P < 0.05$). The index of gluconeogenesis in the exercised rats was not significantly different from controls during recovery.

Figure 1.4: Rates of CO_2 production and clearance during rest, exercise and recovery in rats.
The rates of CO_2 production (rate app, lower curve) and clearance (mcr, upper curve) were determined during two-hour basal (0-120 minutes), four-hour exercise (or rest) (120-360 minutes) and three-hour recovery (360-540 minutes) periods by the constant infusion of $NaH^{14}CO_3$ into rats. Rested rats are represented by the dashed lines, exercised animals by the solid lines. Data are expressed as means $\pm$ SEM. *significantly different from rest ($P < 0.05$). $n = 7$ for exercised rats and $n = 6$ for rested animals.

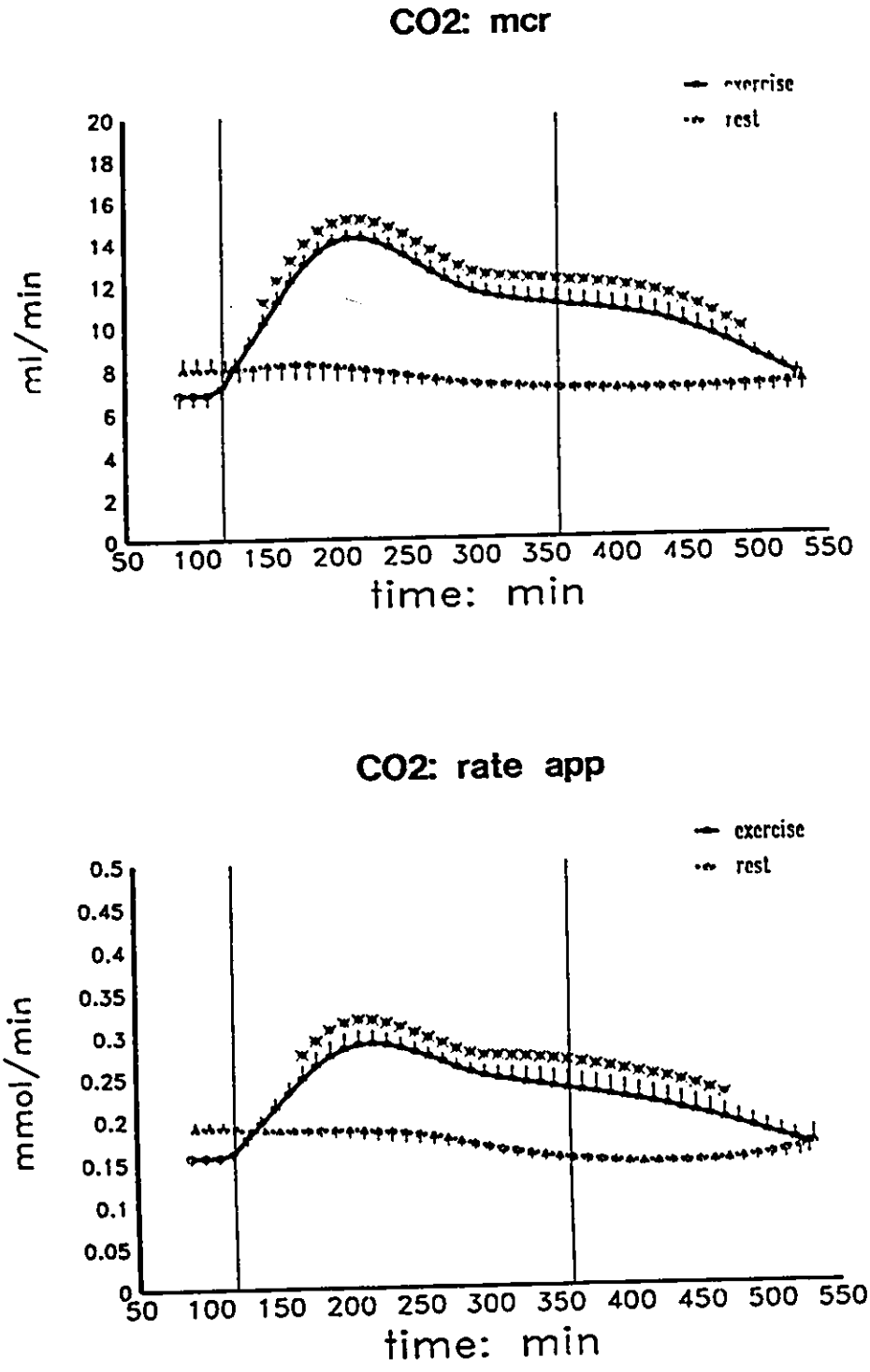

Figure 1.4: Rates of CO_2 production and clearance during rest, exercise and recovery in rats.

CO_2 Clearance and Production (Figure 1.4). There were no significant changes in total plasma CO_2 (Figure 1.1), the rate of CO_2 appearance ($R_{a(CO2)}$) nor in the rate of metabolic clearance of CO_2 (MCR_{CO2}) in rested control animals. $R_{a(CO2)}$ doubled from a resting rate of 0.16 ± 0.01 mmol/min to a peak of 0.29 ± 0.02 mmol/min reached 100 minutes after the onset of exercise. This was also accompanied by a two-fold increase in MCR_{CO2} (6.81 ± 0.47 ml/min to 14.18 ± 0.36 ml/min). By the end of the second hour of recovery, there were no significant differences between exercised or rested animals in either CO_2 production or clearance and basal rates were achieved after three hours of recovery.

Liver glycogen (Figure 1.5). Liver glycogen content was measured at rest (6 hours), immediately after exercise and after a three-hour recovery. Postexercise liver glycogen was virtually depleted (0.26 ± 0.03 mg/g) as compared to fasted rested controls ($P < 0.05$). Hepatic glycogen was 0.79 ± 0.19 mg/g following three hours of postexercise recovery, a value not significantly different than that immediately postexercise.

Figure 1.5: Total liver glycogen in rats after rest, exercise or postexercise recovery. Total liver glycogen was determined in rats following six hours of rest (rest), two hours of rest followed by four hours of exercise (exercise) or two hours of rest followed by four hours of exercise and three hours of postexercise recovery in the fasted state (recovery). Data are expressed as means $\pm$ SEM. *significantly different from rest ($P < 0.05$). $n = 7$ for all rats.

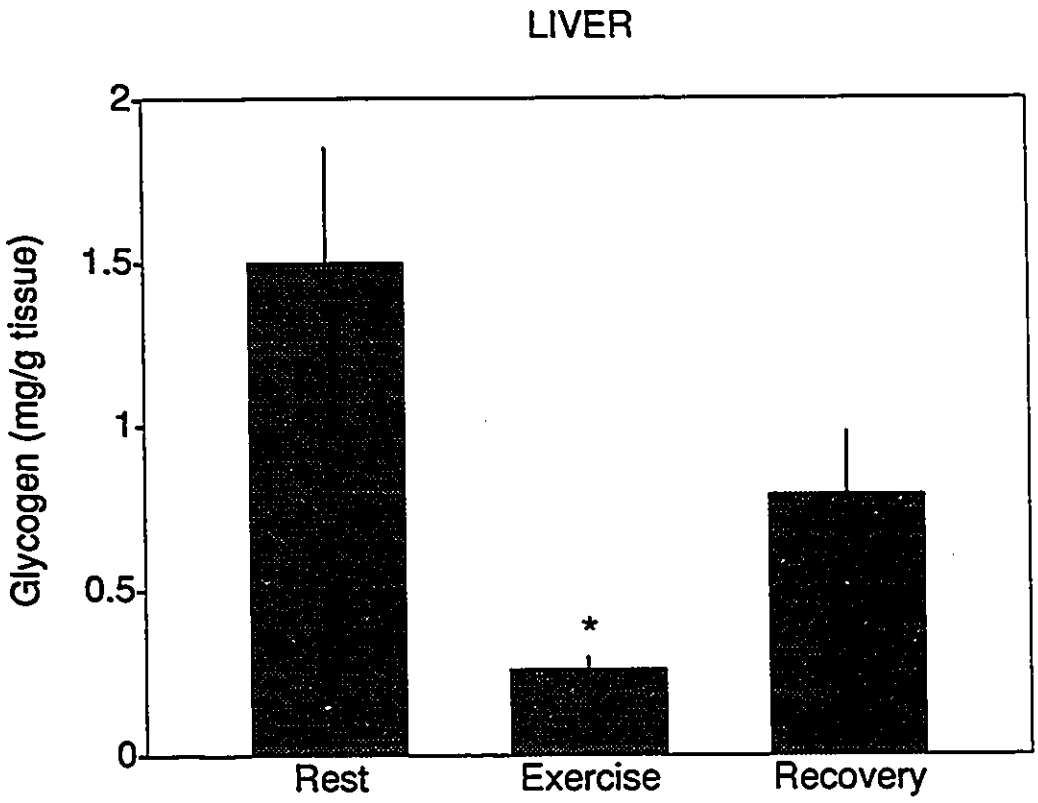

Figure 1.5: Total liver glycogen in rats after rest, exercise or postexercise recovery.

DISCUSSION

Prolonged submaximal exercise (four hours of swimming) in fasted, untrained rats whose liver glycogen is nearly depleted, is characterized by euglycemia during the exercise and an elevation of plasma glucose levels during recovery. This occurs as a consequence of precisely matched increases in glucose production (primarily gluconeogenesis) and glucose utilization during exercise followed by a lag in the decrease of glucose production to basal rates during recovery.

METHODOLOGY

A one-compartment model for glucose kinetics is most appropriate when changes occur slowly. This allows reasonable interpolation between the necessarily few data points obtained during the experimental period (26). The exception to this is during the initial hour following the onset of exercise. The interpolation here may not represent rapid and perhaps transient changes in glucose fluxes which likely occur.

The assessment of gluconeogenic rates by any method (eg. 9, 35) is subject to assumptions and limitations. The merits and assumptions that accompany determination of an index of gluconeogenesis using labelled bicarbonate as the tracer have been discussed previously (27) and have been outlined in the Introduction (pp. 30-31). During exercise, the situation becomes less clear as the specific activity of CO_2 changes (as seen in our data as well as by others, eg. ref. 29) as CO_2 production and compensating ventilation increase. This makes possible only an average estimate

of a gluconeogenic index during the three experimental periods. The changes, although qualitative, give an indication of the relative gluconeogenic fluxes at rest, during exercise and recovery.

Since $^{14}CO_2$ concentrations during a constant infusion of this label were available, CO_2 clearance and production were also calculated. When a one-compartmental model is used in these calculations, the principal assumption is the absence of slow pools of CO_2 which may recycle labelled $^{14}CO_2$ and provide unlabelled CO_2 which is not produced by metabolic pathways.

A partial verification of this approach could be based on other published data on CO_2 production. A resting $\dot{V}_{CO2}$ of 15.0 $\pm$ 0.5 $ml \cdot kg^{-1} \cdot min^{-1}$ has been reported in untrained fasted rats in the weight range of 210 to 240 g (3). This gives a rate of CO_2 production of approximately (15 $ml \cdot kg^{-1} \cdot min^{-1}$ x 0.225 kg)/22.4 $ml \cdot mmol^{-1}$ = 0.15 $\pm$ 0.005 $mmol \cdot min^{-1}$, where 22.4 ml is the volume of 1 mmol of gas at standard temperature and pressure and 0.225 kg is the average weight of the rats. This coincides closely with the average resting production rate of CO_2 in our studies in untrained rats of similar weight (n = 27) of 0.16 $\pm$ 0.01 $mmol \cdot min^{-1}$.

GLUCOSE TURNOVER

These studies demonstrate that untrained fasted animals can maintain glucose homeostasis during prolonged submaximal exercise due to the matched increases in the rates of glucose production and clearance. Others have reported very mild (10) to severe hypoglycemia (5, 32, 33) in rats immediately following prolonged

swimming programs lasting approximately 3-4 hours. The discrepancies are most likely due to the fact that each of our rats swam alone (albeit with continuous agitation) and may not have exercised as vigourously as those swimming "competitively", as it were, in the presence of several animals. Secondly, the latter may invoke additional psychological stress factors which may affect the hormonal profile (catecholamines, glucagon, insulin), increasing the uptake of glucose beyond the rate of production to the point of hypoglycemia.

The intensity of exercise is of pivotal importance to a discussion of glucose turnover as studies have shown that higher intensity exercise is associated with different patterns of glucose production, utilization and regulation compared to low intensity exercise (40% maximal oxygen uptake and no increases in plasma lactate concentrations) (6). Treadmill-run rats show postexercise lactate concentrations from 1.9 mM (8) to 3.5 mM (29) and 4.6 mM (8) for short-term easy, moderate and hard exercise, respectively. Similarly to previous studies (10), lactate concentrations in the swimming rats in this study were 2.4 times higher than basal (1.88 mM) after the first hour of exercise, before declining to 1.3 mM by the fourth hour. Because blood samples were taken only after each hour of exercise, there may have been a larger initial increase of plasma lactate at the onset of exercise which diminished by 60 minutes (as seen in fasted running rats (37)) and thus remained obscure. Our swimming model would therefore qualify as a low- to moderate-intensity exercise.

While our prolonged swimming protocol induced matched two-fold increases in R_a and MCR to maintain plasma glucose concentrations, treadmill running in rats

has induced states of hyperglycemia (28, 31, 34, 35), hypoglycemia (3, 9, 12, 13, 38) or euglycemia (3, 39). Donovan and Sumida (9) report that while R_a in untrained rats doubled during the first 30 minutes of running, the resulting hypoglycemia was attributed to the marked differences in R_a and R_d (rate of glucose disappearance). They also report that the ability of trained rats to avoid hypoglycemia in exercise is due to the increased rate of glucose production and not uptake or clearance of glucose.

Of perhaps equal importance in the effects of exercise intensity on glucose turnover is the preexercise nutritional status. Sonne et al. demonstrated not only does hepatic R_a vary directly with exercise intensity (29), but also that the degree of imbalance between the increases in R_a and MCR are closely related to nutritional status (31). They also suggested that the increased exercise-induced glucose production is just coincidentally better able to match the increase in glucose clearance in fasted compared to fed rats (31). It is suggested that the 12-hour fast used in the present study may prevent hypoglycemia since liver glycogen stores are depleted and gluconeogenesis and fatty acid oxidation may be induced to a greater degree prior to exercise than in, for example, food restricted rats (5).

The rates of glucose production and clearance returned to basal after three hours of postexercise recovery; however, during this recovery period, our rats showed deviation from the seemingly tight control over glucose homeostasis seen during the exercise period. Plasma glucose rose significantly as the rate of decline of R_a lagged behind that of MCR. This is similar to the rebound increases in plasma

glucose reported during recovery from moderate running in rats (29) and intense cycling in man (4). It is consistent with the "feedforward" concept of glucose production - that the exercise-induced increases in R_a are not secondary to increases in glucose uptake nor elicited by muscle substrate need (29) but are probably stimulated by feedforward mechanisms initiated in the central nervous system and, in part, by epinephrine (31).

GLUCONEOGENESIS

Upon examination of the amount of glucose produced over the four-hour exercise (and three-hour recovery) period, the question as to the supply of substrate to support this increased glucose production becomes an issue. Presumably, the first source of glucose would be liver glycogen; however, one would expect a significant depletion of this source following the 12-hour overnight fast (Figure 1.5). Exercise was estimated to have depleted approximately 1.25 mg/g of liver glycogen. Assuming that the liver weight is 4% of the total body weight of the rat (34), this represents approximately 9.25 mg of glycogen. Clearly, with a peak rate of glucose production of 3.58 $\pm$ 0.21 mg/min during exercise, liver glycogen could not have supported more than a few minutes of the increased production of glucose. Gluconeogenesis, then, becomes the key contributor to hepatic glucose output.

Based on the rate of ^{14}C-glucose production divided by the mean CO_2 specific activity during the exercise period, the average calculated gluconeogenic rate is 1.8 mg/min, very nearly half of the total glucose production rate (3.5 mg/min, Figure

1.2). It has been demonstrated, however, that metabolic exchange of ^{14}C for ^{12}C occurs between the gluconeogenic pathway and the Krebs cycle, in the oxaloacetate pool (16). In fasting rats, this dilution factor has been estimated to be 1.4 - 1.5 (16, 17). This yields a relative flux, y, through the gluconeogenic pathway of 0.9 to 1.25 (16, 17). When $^{14}CO_2$ is used to label the gluconeogenic process, this translates into a dilution factor ((1+y)/y, ref. 25) of 1.8 to 2.1. This is consistent with the hypothesis that gluconeogenesis is the only process contributing to glucose production under these circumstances.

It should be noted that the gluconeogenic contributions by glycerol are not included in the above calculations since glycerol is not labelled with $^{14}CO_2$ as it enters the pathway beyond the pyruvate-carboxylation step. This may result in an underestimation of the gluconeogenic index, especially during exercise. However, although blood glycerol concentrations are elevated in fasted rats during prolonged swimming (from 0.05 mM at rest to 0.20 mM at exhaustion, ref. 10), the increase may not be quantitatively important in terms of providing additional gluconeogenic substrate to liver.

Lactate is elevated throughout most of the study and is likely an important substrate for the gluconeogenic process. Alanine, glutamine and glycerol are three other potentially important sources of substrates, particularly during prolonged exercise (eg. 36). It has been suggested that these gluconeogenic substrates are released from muscle, particularly in the form of alanine, for transport to the liver (eg. 11). Indeed, increased gluconeogenic substrate release from muscle has been

reported in fasting rats (14, 23), accompanied by decreases in plasma alanine concentrations (23), suggesting that extraction by the liver exceeds muscle substrate output in periods of high gluconeogenic rates induced by fasting. The situation in exercise, however, is less clear. While similar decreases in plasma alanine have been reported following exhaustive swimming in rats (10), suggestive of elevated hepatic uptake, no alanine efflux from muscle was reported following maximal exercise in humans (16).

FUEL UTILIZATION

Carbon dioxide turnover can be a reflection of fuel utilization during exercise - a relatively higher rate indicative of a greater dependence on carbohydrate oxidation. Rats running at low intensity (13.4 m/min, 1% grade) show the same two-fold increase (from 15.0 to 31.0 $ml \cdot kg^{-1} \cdot min^{-1}$) in the rate of CO_2 production after the first hour of exercise (3) as did our swimming rats, while those running at a higher intensity (26.8 m/min, 1% grade) demonstrate a three-fold rise in CO_2 production (45.7 $ml \cdot kg^{-1} \cdot min^{-1}$) (3). This suggests that lower intensity exercise demonstrates a higher reliance on FFA as fuel, consistent with higher plasma FFA in untrained rats swimming to exhaustion (32) than following exhaustive running at moderate intensity (38).

These observations are also consistent with the data presented here. The doubling of CO_2 production in conjunction with the two-fold increases in glucose production and clearance as well as the plasma concentration of lactate suggest that

during the first 100 minutes of swimming, there is a relatively higher dependence on glucose for fuel than during the latter stages of the exercise. It is interesting to observe that MCR and R_a of glucose reached peak values approximately 80 minutes into the exercise period and then decreased somewhat - in parallel with changes in the production of CO_2. The fall in both fluxes during the latter part of the exercise period is again consistent with a relatively greater reliance of FFA as fuel.

HEPATIC GLYCOGEN

The maintenace of the elevated rates of glucose production well into the recovery period would suggest a replenishing of substrate stores. Yet, while it has been reported (12, 13) that there is no appreciable postexercise glycogen resynthesis in liver so as to preferentially restore muscle glycogen (owing to a hormonal milieu conducive to hepatic glucogeneogenesis rather than glycogenesis), there was some (albeit very little) liver glycogen synthesized during the three hour recovery period in our rats. It has been suggested that the most potent stimulation for glycogen synthesis in liver by glycogen synthase is low concentrations of glycogen (5). The significantly lower postexercise liver glycogen concentrations in the swimming rats in this study (0.26 ± 0.04 mg/g) as compared to those run to exhaustion (eg. 0.74 ± 0.12 mg/g, ref. 12) may stimulate an initial rapid rate of glycogen synthesis until a critical concentration is reached. Indeed, after three hours of postexercise recovery, liver glycogen in the swimming rats (0.79 ± 0.19 mg/g) was similar to that of the running rats (0.91 ± 0.14 mg/g, ref. 12). This increase, however, amounts to only about

4 mg of glucose made during the recovery period. One report of significant hepatic glycogen repletion in rats previously swum to exhaustion (from 0.76 to 5.36 mg/g glycogen), presumably owing to a different hormonal profile (i.e. fivefold lower catcholamine concentrations compared to rats run to exhaustion) (10), is not in keeping with other findings (in running rats, eg. ref. 12, 13), as well as the present study.

Because the rates of glucose production remain elevated in recovery, and since this glucose is apparently not being used to replete liver glycogen, it is suggested that this excess plasma glucose is used for the preferential replenisment of muscle glycogen following prolonged swimming.

REFERENCES

1. Ahlborg, G., P. Felig, L. Hagenfeldt, R. Hendler, and J. Wahren. Substrate turnover during prolonged exercise in man. J. Clin. Invest. 53: 1080-1090, 1974.

2. Ahlborg, G., and P. Felig. Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. J. Clin. Invest. 69: 45-54, 1982.

3. Brooks, G. A., and C. M. Donovan. Effect of endurance training on glucose kinetics during exercise. Am. J. Physiol. 244 (Endocrinol. Metab. 7): E505-E512, 1983.

4. Calles, J., J. J. Cunningham, L. Nelson, N. Brown, E. Nadel, R. S. Sherwin, and P. Felig. Glucose turnover during recovery from intensive exercise. Diabetes 32: 734-738, 1983.

5. Conlee, R. K., R. C. Hickson, W. W. Winder, J. M. Hagberg, and J. O. Holloszy. Regulation of glycogen resynthesis in muscles of rats following exercise. Am. J. Physiol. 235 (Regulatory Integrative Comp. Physiol. 4): R145-R150, 1978.

6. Cooper, D. M., T. J. Barstow, A. Bergner, and W. N. P. Lee. Blood glucose turnover during high- and low-intensity exercise. Am. J. Physiol. 257 (Endocrinol. Metab. 20) E:405-E412, 1989.

7. deBodo, R. C., R. Steele, N. Altszuler, A. Dunn, and J. S. Bishop. On the hormonal regulation of carbohydrate metabolism, studies with ^{14}C-glucose. Recent Prog. Horm. Res. 19: 445-482, 1963.

8. Donovan, C. M., and G. A. Brooks. Endurance training affects lactate clearance, not lactate production. Am. J. Physiol. 244 (Endocrinol. Metab. 7): E83-E92, 1983.

9. Donovan, C. M., and K. D. Sumida. Training improves glucose homeostasis in rats during exercise via glucose production. Am. J. Physiol. 258 (Regulatory Integrative Comp. Physiol. 27): R770-R776, 1990.

10. Favier, R. J., H. E. Koubi, M. H. Mayet, B. Semporé, B. Simi, and R. Flandrois. Effects of gluconeogenic precursor flux alterations on glycogen resynthesis after prolonged exercise. J. Appl. Physiol. 63(5): 1733-1738, 1987.

11. Felig, P. The glucose-alanine cycle. Metabolism 22: 179-207, 1973.

12. Fell, R. D., J. A. McLane, W. W. Winder, and J. O. Holloszy. Preferential resynthesis of muscle glycogen in fasting rats after exhausting exercise. Am. J. Physiol. 238 (Regulatory Integrative Comp. Physiol. 7): R328-R332, 1980.

13. Gaesser, G. A., and G. A. Brooks. Glycogen repletion following continuous and intermittent exercise to exhaustion. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(4): 722-728, 1980.

14. Goodman, M. N., R. Dietrich, and P. Luu. Formation of gluconeogenic precursors in rat skeletal muscle during fasted-refed transition. Am. J. Physiol. 49(4): 722-728, 1980.

15. Hassid, W. Z., and S. Abraham. Chemical procedures for analysis of polysaccharides. In Methods in Enzymology, Vol. III (S. P. Colowick and N. O. Kaplan, eds.) Academic Press, New York, N.Y., pp. 34-50, 1957.

16. Hermansen, L. and O. Vaage. Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. Am J. Physiol. 233(5): E422-E429, 1977.

17. Hetenyi, G., Jr. Correction for the metabolic exchange of ^{14}C for ^{12}C atoms in the pathway of gluconeogenesis *in vivo*. Fed. Proc. 41: 104-109, 1982.

18. Hetenyi, G., Jr., B. Lussier, C. Ferrarotto, and J. Radziuk. Calculation of the rate of gluconeogenesis from the incorporation of ^{14}C atoms from labelled bicarbonate or acetate. Can. J. Physiol. Pharmacol. 60: 1603-1609, 1982.

19. Huskisson, N. S., and P. F. V. Ward. A reliable method for scintillation counting of $^{14}CO_2$ trapped in solutions of sodium hydroxide, using a scintillant suitable for general use. Inter. J. Appl. Radiat. Isotopes 29: 729-734, 1978.

20. Issekutz, B., Jr., W. A. S. Shaw, and A. C. Issekutz. Lactate metabolism in resting and exercising dogs. J. Appl. Physiol. 40(3): 312-319, 1976.

21. John-Alder, H. B., R. M. McAllister, and R. L. Terjung. Reduced running endurance in gluconeogenesis-inhibited rats. Am. J. Physiol. 251 (Regualtory Integrative Comp. Physiol. 20): R137-R142, 1986.

22. Jones, J. K. N., and R. J. Stoodley. Determination of isotopic carbon distribution in aldoses. In Methods in Carbohydrate Chemistry, VII (R. L. Whistler and M. L. Wolfrom, eds.) Academic Press, New York, N.Y., pp. 489-509, 1963.

23. MacDonald, M., N. Neufeldt, B. N. Park, M. Berger, and N. Ruderman. Alanine metabolism and gluconeogenesis in the rat. Am. J. Physiol. 231(2): 619-625, 1976.

24. Passoneau, J. V. L-(+)-Lactate: determination with lactate dehydrogenase and NAD. In Methods of Enzymatic Analysis (H. -U. Bergmeyer, ed.) Academic Press, New York, N. Y., pp. 1468-1474, 1974.

25. Radziuk, J. Sources of carbon in hepatic glycogen synthesis during absorption of an oral glucose load in humans. Fed. Proc. 41: 110-116, 1982.

26. Radziuk, J., K. H. Norwich, and M. Vranic. Experimental validation of measurements of glucose turnover in nonsteady state. Am. J. Physiol. 234(1): E84-E93, 1978.

27. Radziuk, J. Hepatic glycogen in humans. II. Gluconeogenetic formation after oral and intravenous glucose. Am. J. Physiol. 257: E158-E169, 1989.

28. Somogyi, M. Notes on sugar determination. J. Biol. Chem. 195: 19-23, 1952.

29. Sonne, B., and H. Galbo. Carbohydrate metabolism during and after exercise in rats: studies with radioglucose. J. Appl. Physiol. 59(5): 1627-1639, 1985.

30. Sonne, B., and H. Galbo. Carbohydrate metabolism in fructose-fed and food-restricted running rats. J. Appl. Physiol. 61(4): 1457-1466, 1986.

31. Sonne, B., K. J. Mikines, and H. Galbo. Glucose turnover in 48-hour-fasted running rats. Am. J. Physiol. 252 (Regulatory Integrative Com. Physiol. 21): R587-R593, 1987.

32. Terblanche, S. E., R. D. Fell, A. C. Juhlin-Dannfelt, B. W. Craig, and J. O. Holloszy. Effects of glycerol feeding before and after exhausting exercise in rats. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 50(1): 94-101, 1981.

33. Terjung, R. L., K. M. Baldwin, P. A. Molé, G. H. Klinkerfuss, and J. O. Holloszy. Effect of running to exhaustion on skeletal muscle mitochondria: a biochemical study. Am. J. Physiol. 223(3): 549-554, 1972.

34. Vissing, J., B. Sonne, and H. Galbo. Role of metabolic feedback regulation in glucose production of running rats. Am. J. Physiol. 255 (Regulatory Integrative Comp. Physiol. 24): R400-R406, 1988.

35. Vissing, J., J. L. Wallace, and H. Galbo. Effect of liver glycogen content on glucose production in running rats. J. Appl. Physiol. 66(1): 318-322, 1989.

36. Wasserman, D. H., and A. D. Cherrington. Hepatic fuel metabolism during muscular work: role and regulation. Am. J. Physiol. 260: E811-E824, 1991.

37. Winder, W. W., M. A. Beattie, and R. T. Holman. Endurance training attenuates stress hormone responses to exercise in fasted rats. Am. J. Physiol. 243 (Regulatory Integrative Comp. Physiol. 12): R179-R184, 1982.

38. Winder, W. W., R. T. Holman, and S. J. Garhart. Effect of endurance training on liver cAMP response to prolonged submaximal exercise. Am. J. Physiol. 240 (Regulatory Integrative Comp. Physiol. 9): R330-R334, 1981.

39. Winder, W. W., M. L. Terry, and V. M. Mitchell. Role of plasma epinephrine in fasted exercising rats. Am. J. Physiol. 248 (Regulatory Integrative Comp. Physiol. 17): R302-R307, 1985.

CHAPTER 2: PATHWAYS OF POSTEXERCISE MUSCLE AND LIVER GLYCOGEN SYNTHESIS

INTRODUCTION

The maintenance of the elevated rates of postexercise glucose production without significant amounts of hepatic glycogen formation (as seen in Chapter 1) suggests the preferential repletion of muscle glycogen even in the absence of exogenous substrate as reported previously (16, 17, 27). The pattern of such postexercise glycogen repletion in terms of the source or sources of muscle glycogen, be it entirely plasma glucose (direct route) or partially glucogenic precursors such as lactate (glyconeogenic route), has not been clearly established. Evidence is accumulating to suggest that the indirect pathway could be a significant contributor to glycogenesis in frog (6, 12), rabbit (6, 32), lizard (18), rat (24, 28, 38), mouse (8, 40) and human (5, 20) muscle.

Johnson and Bagby (24) have reported that prior exercise (treadmill running) does not affect the pathways of tissue glycogen repletion and that glyconeogenesis can occur in white muscle, but only in the presence of high lactate levels produced by nutritive support. Stevenson et al. (39) also reported glyconeogenesis in perfused rat hindlimbs, but only with high concentrations of lactate in the perfusate. Prolonged swimming, however, is characterized not only by significant muscle glycogen depletion (13, 15, 39, 42, 43) and substantial postexercise repletion during fasted recovery (15), but also by minimal plasma lactate elevation (13, 15, Chapter 1). We used this swimming model, then, to investigate whether muscle glycogen was repleted

to any extent via the indirect pathway given the apparent lack of glyconeogenic substrate, or whether plasma glucose could account for all of the glycogen synthesized during the postexercise recovery period.

METHODS

EXPERIMENTAL PROTOCOL

The experimental design used was identical to the one described in Chapter 1 with the following exceptions. Three series of swimming and resting rats were studied; the first two series (I and II) were those described Chapter 1 which were infused with $NaH^{14}CO_3$ and D-[^{3}H-6]-glucose and sacrificed either immediately following the four-hour exercise or rest period (I) or after the three-hour postexercise recovery period (II); the third series of rats (III) was infused with [^{14}C-U]-lactate along with D-[^{3}H-6]-glucose throughout the nine-hour protocol and sacrificed following the recovery period. The priming dose for [^{14}C-U]-lactate was approximately 3.94 μCi which was followed by a constant intravenous infusion of approximately 0.03 $\mu Ci \cdot min^{-1}$; the other tracers were infused at rates described previously (Chapter 1). At the end of the study, in addition to the liver, three muscles of the hindlimb - soleus, white and red gastrocnemii (representative of slow-twitch oxidative, fast-twitch glycolytic and fast-twitch oxidative glycolytic muscle fibers, respectively) - were freeze-clamped for the assessment of total and radiolabelled glycogen.

CHEMICAL ASSAYS

Analyses of plasma samples for total and radiolabelled (^{3}H and ^{14}C) glucose in Series I and II were described in Chapter 1. ^{3}H- and ^{14}C-glucose of Series III plasma samples were determined directly from the column eluate fractions without potassium gluconate formation. Muscles from both hindlimbs were combined into one sample for each of the three different muscle types for each rat; total glycogen in each of the three muscles was determined as described for that of liver glycogen except that the volumes of ethanol precipitation and washings were one-third of those used for liver samples. A 1 ml aliquot of the final 2 ml hydrolyzed glycogen solution was counted directly for the determination of ^{3}H- and ^{14}C-glucose in muscle and liver glycogen.

CALCULATIONS

Tissue glycogen synthesized from direct uptake of plasma glucose was calculated by

$$direct\ synthesis = \frac{[^3H]\text{-}d.p.m.\ tissue\ glycogen}{SA\ plasma\ [^3H]\text{-}glucose} \qquad (2.1)$$

where *[^{3}H]-d.p.m.* are those from ^{3}H-glucose in tissue glycogen and *SA* is the average specific activity of ^{3}H-glucose in plasma (C*/C) during recovery (or during exercise for rats sacrificed immediately postexercise). Gluconeogenic formation of glycogen was indexed by comparing the total amount of ^{14}C-glycogen in tissue to that originating from plasma ^{14}C-glucose during the recovery period. ^{14}C-glycogen in the tissues which could be accounted for by the direct uptake of ^{14}C-glucose in plasma was

calculated by

$$direct\ [^{14}C]\text{-}glycogen = \frac{tissue\ [^{3}H]\text{-}glycogen\ d.p.m.}{plasma\ \frac{[^{3}H]\text{-}glucose\ d.p.m.}{[^{14}C]\text{-}glucose\ d.p.m.}} \quad (2.2)$$

where the ratio of ^{3}H- to ^{14}C-glucose d.p.m. in plasma are averaged during the recovery period. An index of the amount of glycogen formed by the indirect or glyconeogenic pathway was calculated by

$$index = \frac{total\ [^{14}C]\text{-}glycogen\ -\ direct\ [^{14}C]\text{-}glycogen}{SA\ plasma\ [^{14}C]\text{-}precursor} \quad (2.3)$$

where *total ^{14}C-glycogen* is the total amount of ^{14}C-d.p.m. in tissue glycogen, *direct ^{14}C-glycogen* is that calculated from equation 2.2 and *SA* is the average plasma specific activity of the precursor tracer (i.e. $^{14}CO_2$ in Series I and II and ^{14}C-lactate for Series III) during recovery.

STATISTICAL ANALYSIS

Significant differences ($P < 0.05$) between the total ^{14}C-d.p.m. in muscle and liver glycogen and that which could be accounted for by the direct uptake of ^{14}C-glucose from plasma were detected using a modification of the statistical methods applied for the detection of minimal detectable doses in radioligand assays (36). Theoretically, there should be no incorporation of ^{14}C from $^{14}CO_2$ into muscle glycogen via glyconeogenesis in rats infused with $NaH^{14}CO_3$ (22) likely due to the lack of adequate equilibration in the fumarase reaction in muscle, resulting in the loss

of the label (7); hence, there should be no differences between total and direct ^{14}C-glycogen in these rats. The differences between total *versus* direct ^{14}C-glycogen that are observed in $NaH^{14}CO_3$-infused rats, then, were due to the sensitivities of the assays, associated errors in the calculations and/or noncompliance with the assumption of linear glycogen formation during the period examined. Thus, these differences can be used to define a statistical "zero" value or minimal limit, below which any differences observed in rats infused with [^{14}C-U]-lactate would be considered not statistically meaningful beyond the errors of the methods. The ratios of ^{3}H-glucose to ^{14}C-glucose in $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats were comparable, justifying the use of the ^{14}C-d.p.m. minimal detectable differences calculated using $NaH^{14}CO_3$-infused rats.

The minimal detectable difference (MDD) is given by

$$MDD = m_o + t \cdot \sigma \sqrt{\frac{1}{n_1} + \frac{1}{n_2}} \qquad (2.4)$$

where m_o is the average of the differences of total and direct ^{14}C-d.p.m. in glycogen in the three muscles in rats infused with $NaH^{14}CO_3$ (Series II), σ is the standard deviation of the average of the differences, n_1 and n_2 are the number of samples used in the determination of the average of the differences and in the difference to be tested, respectively, and t is the percentile in the Student's t distribution with (n_1+n_2-2) degrees of freedom at the 5% level of significance.

Statistical comparisons of the total glycogen levels, glycogen synthesized from glucose (direct synthesis), glycogen from glyconeogenic synthesis, and total ^{14}C-d.p.m. in glycogen within and between Series I rats (no recovery) and Series II rats (recovery) were done using analysis of variance with a completely randomized factorial design and an *a posteriori* comparison by Scheffe's procedure, using the statistical package, SAS. The same comparisons made between rested and exercised rats within Series III were done using Student's *t* tests. The level of significance for all statistical tests was set at $P < 0.05$.

RESULTS

TOTAL TISSUE GLYCOGEN: DEPLETION AND REPLETION

Tissue Glycogen (Figure 2.1).

Rats infused with saline from all studies (Chapters 1, 2 and 3) were included in Figure 2.1 (p. 87) so as to give a better assessment of the degrees of depletion and repletion of tissue glycogen following prolonged exercise (or rest) and recovery.

Liver. The overnight fast and six hours of rest (no recovery) resulted in low but variable liver glycogen concentrations (1.85 ± 0.40 mg/g liver). There was a net depletion of glycogen in the resting animals during three more hours of fasting (recovery period) to a final concentration of 0.71 ± 0.18 mg/g liver, significantly lower than those of rats with no recovery ($P < 0.05$). Exercise depleted the liver to

very low glycogen concentrations (0.28 ± 0.02 mg/g liver) with little variability, representing 85% depletion as compared to the nonexercised controls sacrificed at the same time ($P < 0.05$). During three hours of postexercise recovery, hepatic glycogen rose to 1.01 ± 0.22 mg/g liver ($P < 0.05$).

Soleus muscle. The soleus muscle was not depleted during exercise to any significant degree as compared to rested controls (2.63 ± 0.16 and 3.07 ± 0.20 mg/g muscle, respectively). While there was no net change in total glycogen concentration during recovery in rested control muscles, exercised animals demonstrated a net depletion of soleus glycogen during the three-hour postexercise recovery period giving a final concentration of 1.76 ± 0.17 mg/g, approximately 45% and 43% lower than total glycogen concentrations in both rested controls and those immediately following the swim, respectively ($P < 0.05$).

White gastrocnemius muscle. Total resting fasted glycogen content in white gastrocnemius muscle (5.86 ± 0.34 mg/g muscle) was double that of soleus muscle. There was a slight decrease in total glycogen in resting rats by the end of the recovery period to 4.84 ± 0.40 mg/g. Swimming induced a 47.6 ± 5.1% decrease in glycogen concentration in the muscle (to 3.07 mg/g) as compared to the rested animals ($P < 0.05$). During the first hour of postexercise recovery, there was no apparent net glycogen synthesis in white muscles according to data from preliminary studies of $n = 3$ rats sacrificed at 60 minutes of recovery (data not shown); during

the final two hours of recovery, however, there was a slow rate of repletion giving a final total glycogen content 78.1% of the rested control ($P < 0.05$).

Red gastrocnemius muscle. The red gastrocnemius muscle was depleted 50.4 ± 6.8% immediately following swimming as compared to the rested controls (2.28 ± 0.29 mg/g *versus* 4.60 ± 0.24 mg/g muscle, $P < 0.05$). During the postexercise recovery period, net glycogen synthesis was linear throughout the three-hour recovery period (according to the preliminary studies of $n = 3$ rats sacrificed at one hour recovery, data not shown) and was almost completely repleted (91.7% of rested control) by the end of the three hours (3.84 ± 0.24 mg/g in exercised and 4.19 ± 0.23 mg/g in rested animals). Resting animals showed no significant changes in red gastrocnemius muscle glycogen at 0 and 180 minutes of recovery.

Figure 2.1: Total liver and muscle glycogen in all rested and exercised rats. Total glycogen concentration was measured in liver, soleus and white and red gastrocnemii in rats following 2 hours of rest and either 4 hours of exercise or rest (0 minutes of recovery) ($n = 13$ for both rested and exercised rats) and after 180 minutes of fasted postexercise recovery ($n = 20$) or rest ($n = 18$). Data are expressed as means $\pm$ SEM. Rested rats are represented by the dashed lines, exercised animals by the solid lines. *significantly different from rest, $^{+}$significantly different from 0 min. recovery ($P < 0.05$).

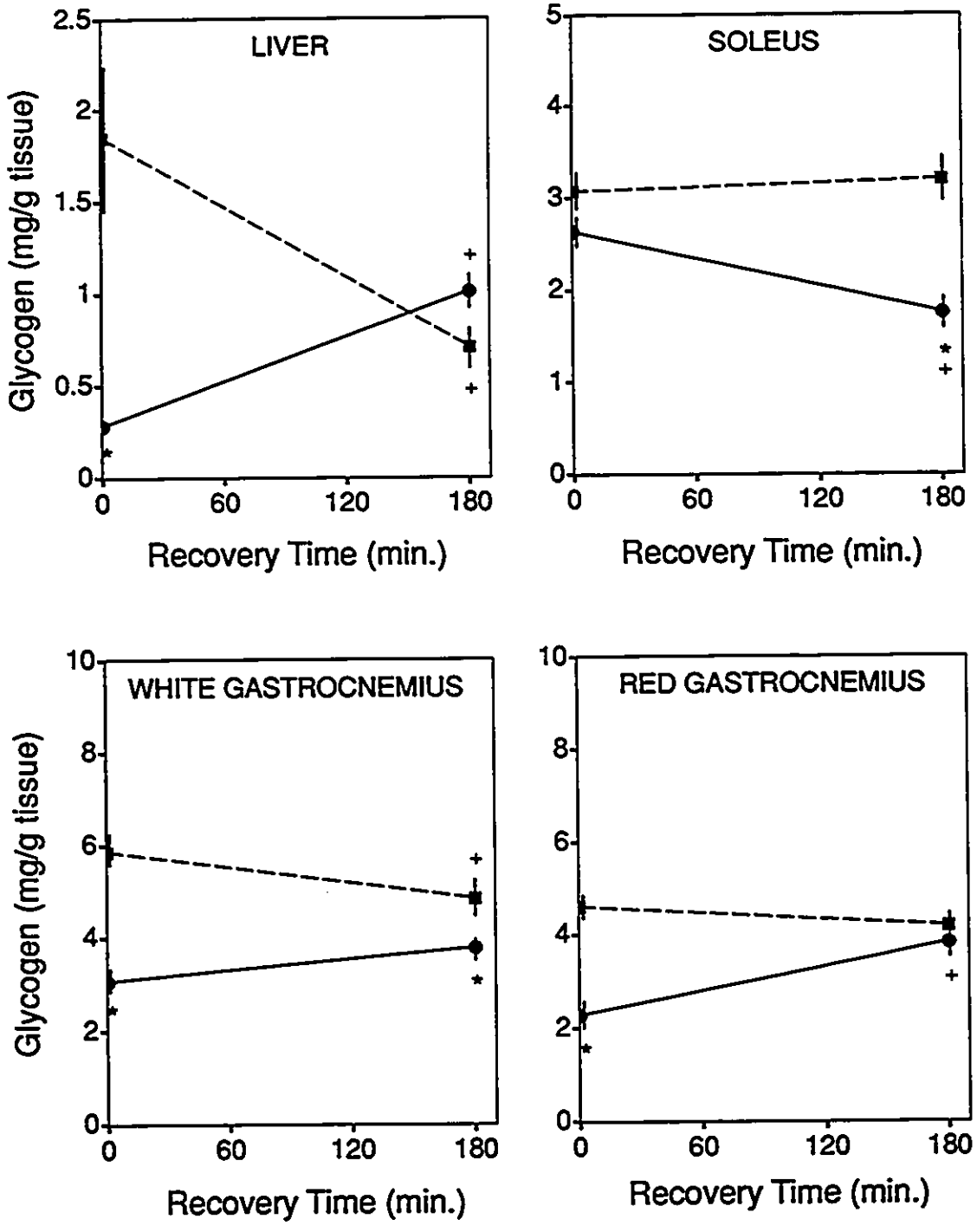

Figure 2.1: Total liver and muscle glycogen in all rested and exercised rats.

Figure 2.2: Liver glycogen in rested and exercised rats infused with [^{3}H-6]-glucose and either $NaH^{14}CO_3$ or [^{14}C-U]-lactate.
Liver glycogen in rats infused with $NaH^{14}CO_3$ and [^{3}H-6]-glucose was assessed immediately following 4 hours of exercise or rest (no recovery, far left panels) and after 3 hours of recovery (recovery, middle panels). Liver glycogen in [^{14}C-U]-lactate-, [^{3}H-6]-glucose-infused rats was assessed after 4 hours of exercise or rest and 3 hours of recovery (recovery, far right panels). Upper panels: open bars are total ^{14}C-glycogen d.p.m. in liver; striped bars are ^{14}C-glycogen d.p.m. in liver accounted for by the direct uptake of ^{14}C-glucose from plasma. Lower panels: open bars are total liver glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis. All data are expressed as means $\pm$ SEM. **significantly different from total ^{14}C-glycogen, *significantly different from rest, $^+$significantly different from no recovery ($P < 0.05$). $n = 7$ for all bars except those of rested rats with recovery where $n = 6$.

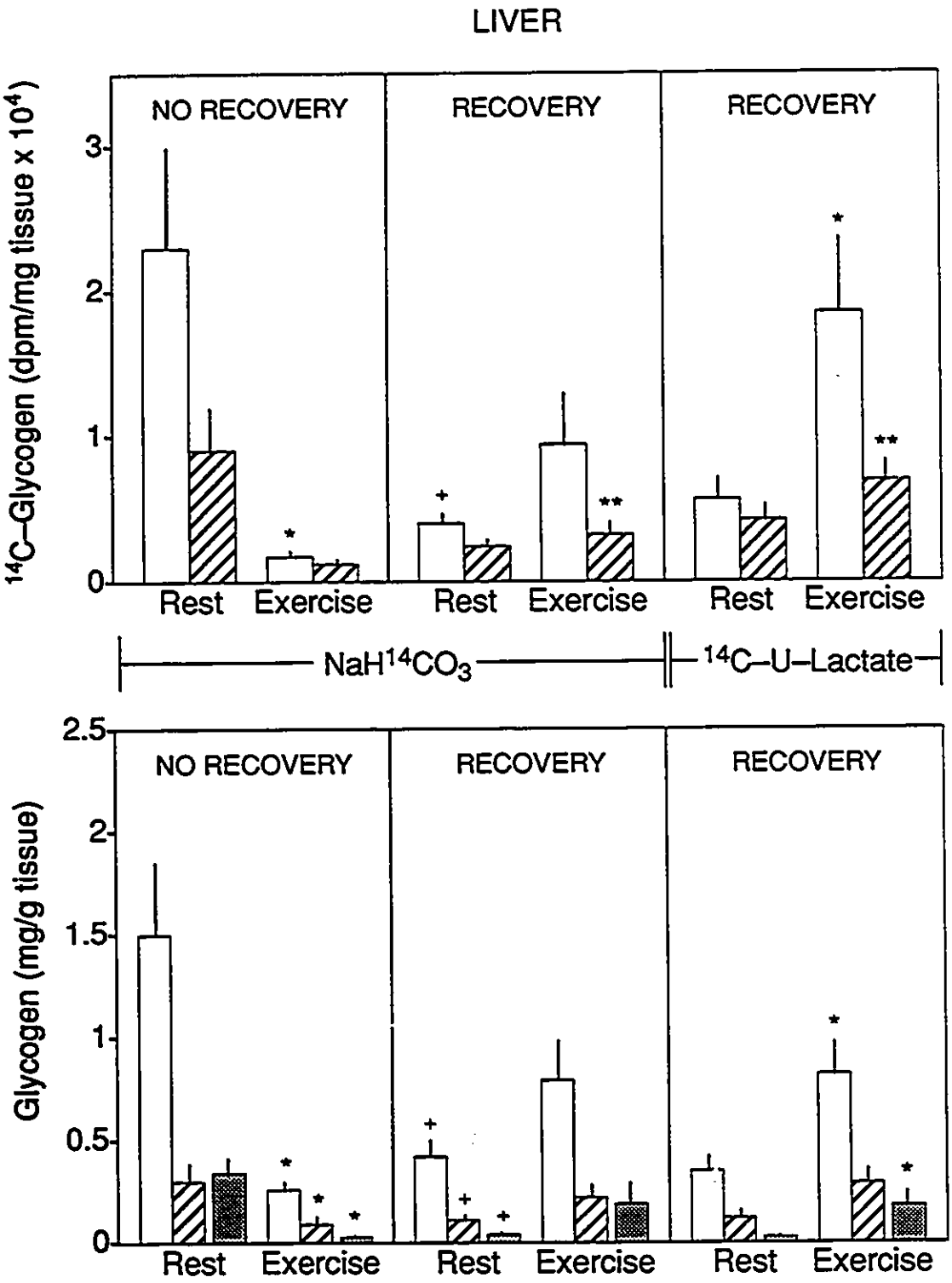

Figure 2.2: Liver glycogen in rested and exercised rats infused with [^{3}H–6]–glucose and either NaH^{14}CO$_3$ or [^{14}C–U]–lactate.

RADIOLABELLED TISSUE GLYCOGEN: DIRECT AND INDIRECT SYNTHESIS

Liver Glycogen (Figure 2.2)

The upper panels of Figure 2.2 show the total d.p.m. of ^{14}C-glycogen in liver and that resulting from the direct uptake of ^{14}C-glucose from plasma following four hours of exercise or rest (far left panel), as well as following a three-hour recovery period, using either $NaH^{14}CO_3$ or [^{14}C-U]-lactate as the tracer (middle and far right panels, respectively). In animals with no recovery, there was a significant decrease in total ^{14}C-glycogen immediately postexercise as compared to rested controls ($P < 0.05$). In rested animals, the three-hour recovery period resulted in a significant decrease in total ^{14}C-glycogen ($P < 0.05$). In [^{14}C-U]-lactate-infused rats, total ^{14}C-glycogen was higher in exercised compared to rested rats ($P < 0.05$).

Total and direct ^{14}C-glycogen were not statistically significantly different following recovery in rested rats. Following recovery (in Series II and III), total ^{14}C-glycogen in liver in exercised rats was more than double that of rested controls while the direct ^{14}C incorporation into glycogen increased only slightly, resulting in significant differences between the total d.p.m. of ^{14}C-glycogen and that which could be accounted for by the direct uptake of plasma ^{14}C-glucose ($P < 0.05$) in exercised rats following recovery with both $NaH^{14}CO_3$ and [^{14}C-U]-lactate as tracers. The corresponding relative glyconeogenic indices (i.e. the percent differences in total *versus* direct ^{14}C-glycogen) rose to 66.0% and 62.7% with $NaH^{14}CO_3$ and [^{14}C-U]-lactate tracers, respectively.

The lower panels of Figure 2.2 show total liver glycogen, glycogen synthesized by the direct uptake of glucose and the indices of glycogen formed by the gluconeogenic pathway in liver in rested and exercised rats without (left panel) and with a three-hour recovery period (middle and right panels). Following four hours of swimming, total liver glycogen was significantly depleted (from 1.50 $\pm$ 0.35 to 0.26 $\pm$ 0.04 mg/g, $P < 0.05$) (lower left panel). Direct glycogen synthesis as well as the index of gluconeogenic glycogen formation were also significantly reduced during the exercise period as compared to nonexercised controls ($P < 0.05$). Three hours of recovery in rested rats resulted in similar significant decreases in total and direct glycogen as well as the index of gluconeogenic glycogen ($P < 0.05$) (lower middle panel). Total and direct glycogen and the index of gluconeogenic glycogen in exercised rats following postexercise recovery (Series II) were significantly different from neither rested control values (middle panel) nor those of rats immediately following exercise (left panel). In [^{14}C-U]-lactate-infused exercised animals (right panel), the postrecovery increases in total glycogen and the index of glyconeogenesis were statistically higher than those of rested controls ($P < 0.05$).

Soleus Glycogen (Figure 2.3)

Although there were no individual statistical differences in total ^{14}C-d.p.m. in glycogen between rested and exercised rats without and with postexercise recovery, there was a main rest/exercise effect ($P < 0.05$) in rats infused with $NaH^{14}CO_3$ (upper left and middle panels), whereby exercise induced a decrease in the incorpor-

ation of ^{14}C into glycogen. The relatively high incorporation of ^{14}C into soleus glycogen at rest compared to that in the gastrocnemius muscles (2.6 times higher than that in the red gastrocnmemius (Figure 2.5) and 15-fold higher than that in the white gastrocnemius (Figure 2.4)) reflects the very high glycogen turnover in the soleus. There were no statistically significant differences between total ^{14}C-glycogen and that accounted for by the direct uptake of ^{14}C-glucose from plasma in [^{14}C-U]-lactate-infused rested or exercised rats, suggesting that all of the glycogen was made via the direct pathway in the soleus.

The lack of any significant differences between total and direct ^{14}C-glycogen is reflected in the very low indices of glyconeogenesis (lower panels, negative values not depicted on graphs). Total glycogen in soleus was significantly lower in exercised rats compared to rested controls following recovery (2.19 $\pm$ 0.39 *versus* 3.32 $\pm$ 0.45 mg/g muscle in $NaH^{14}CO_3$-infused rats and 1.65 $\pm$ 0.17 *versus* 3.23 $\pm$ 0.47 mg/g muscle in [^{14}C-U]-lactate-infused rats, $P < 0.05$) (Figure 2.3, lower panels) even though glycogen was not depleted in the muscle at the end of the prolonged swim (left panel), indicating net glycogen depletion during the recovery period. Soleus glycogen synthesized by the direct uptake of plasma glucose was not different immediately following exercise or rest (1.29 $\pm$ 0.21 and 1.18 $\pm$ 0.36 mg/g, respectively) (left panel), nor was it different in rested or exercised rats following recovery (middle and right panels). The levels of direct glycogen synthesis in rested and exercised animals again reflect the high turnover of glycogen in the soleus muscle.

Figure 2.3: Soleus glycogen in rested and exercised rats infused with [^{3}H-6]-glucose and either $NaH^{14}CO_3$ or [^{14}C-U]-lactate.
Soleus glycogen in rats infused with $NaH^{14}CO_3$ and [^{3}H-6]-glucose was assessed immediately following 4 hours of exercise or rest (no recovery, far left panels) and after 3 hours of recovery (recovery, middle panels). Soleus glycogen in [^{14}C-U]-lactate-, [^{3}H-6]-glucose-infused rats was assessed after 4 hours of exercise or rest and 3 hours of recovery (recovery, far right panels). Upper panels: open bars are total ^{14}C-glycogen d.p.m. in soleus; striped bars are ^{14}C-glycogen d.p.m. in soleus accounted for by the direct uptake of ^{14}C-glucose from plasma. Lower panels: open bars are total soleus glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis (negative values do not appear on the graphs). All data are expressed as means $\pm$ SEM. *significantly different from rest ($P < 0.05$). $n = 7$ for all bars except those of rested rats with recovery where $n = 6$.

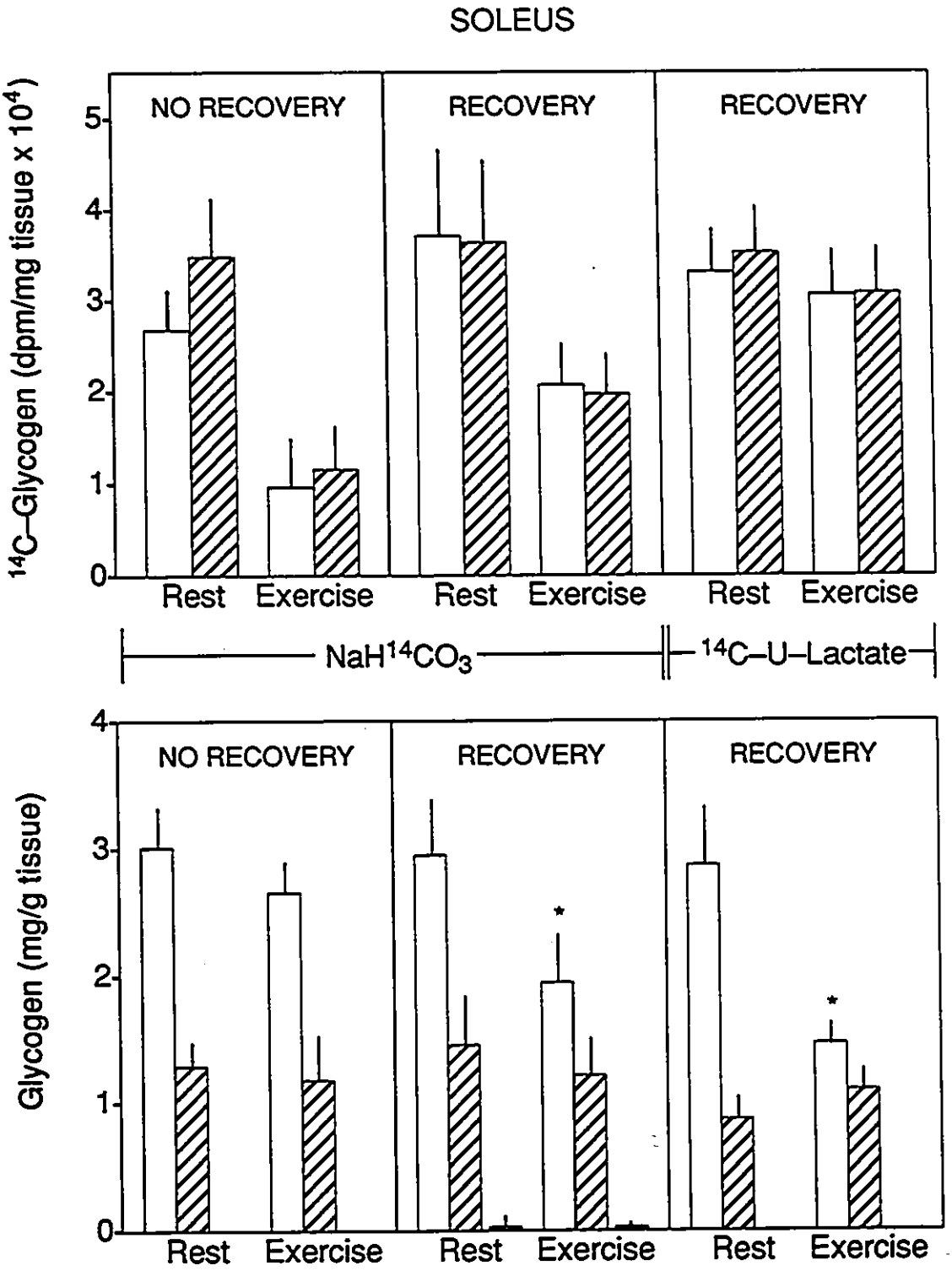

Figure 2.3: Soleus glycogen in rested and exercised rats infused with [^{3}H–6]–glucose and either $NaH^{14}CO_3$ or [^{14}C–U]–lactate.

Figure 2.4: White gastrocnemius glycogen in rested and exercised rats infused with [^{3}H-6]-glucose and either $NaH^{14}CO_3$ or [^{14}C-U]-lactate.
White gastrocnemius glycogen in rats infused with $NaH^{14}CO_3$ and [^{3}H-6]-glucose was assessed immediately following 4 hours of exercise or rest (no recovery, far left panels) and after 3 hours of recovery (recovery, middle panels). White gastrocnemius glycogen in [^{14}C-U]-lactate-, [^{3}H-6]-glucose-infused rats was assessed after 4 hours of exercise or rest and 3 hours of recovery (recovery, far right panels). Upper panels: open bars are total ^{14}C-glycogen d.p.m. in white gastrocnemius; striped bars are ^{14}C-glycogen d.p.m. in white gastrocnemius accounted for by the direct uptake of ^{14}C-glucose from plasma. Lower panels: open bars are total white gastrocnemius glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis (negative values do not appear on the graph). All data are expressed as means $\pm$ SEM. **significantly different from total ^{14}C-glycogen, *significantly different from rest, $^+$significantly different from no recovery ($P < 0.05$). $n = 7$ for all bars except those of rested rats with recovery where $n = 6$.

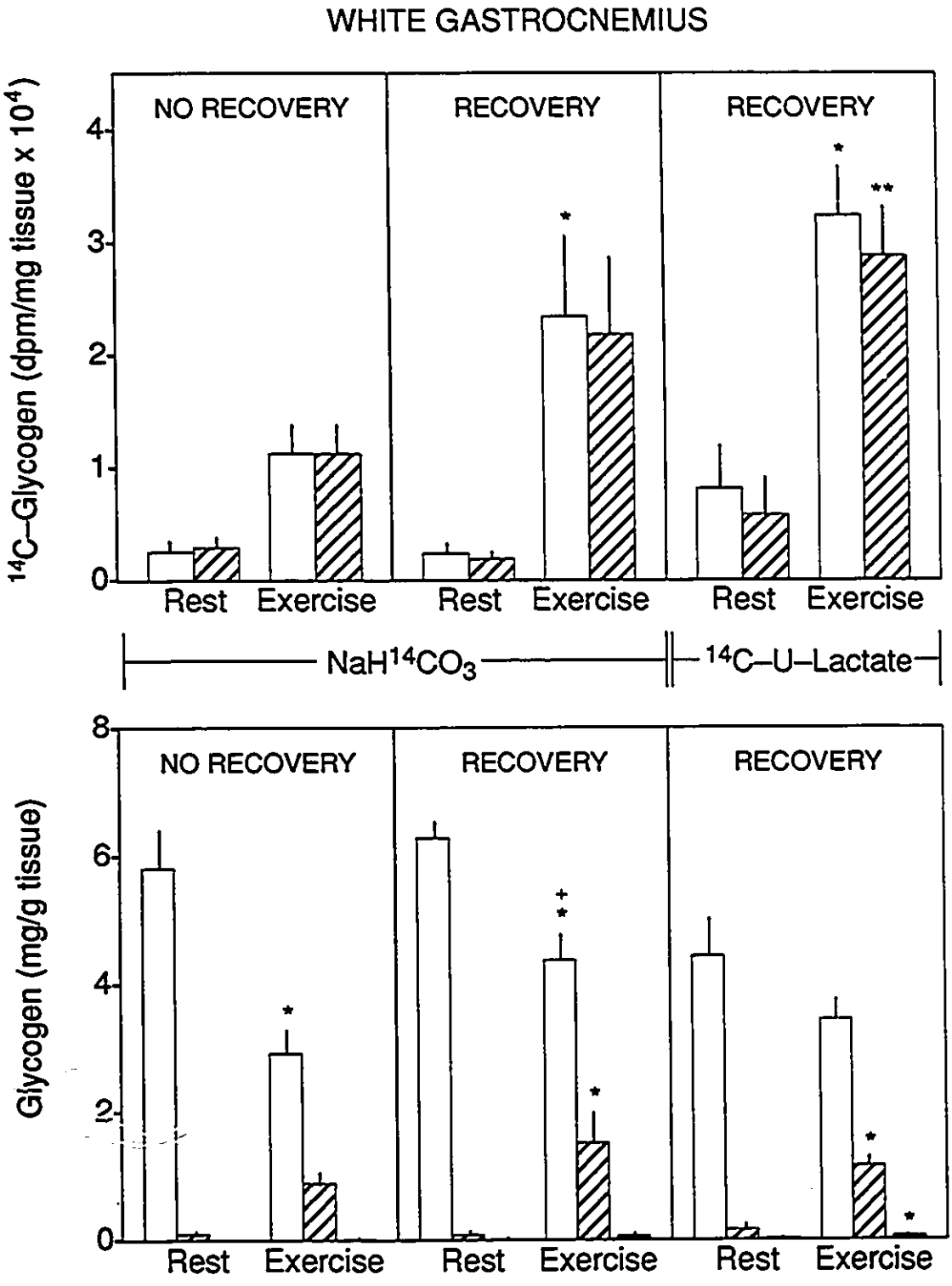

Figure 2.4: White gastrocnemius glycogen in rested and exercised rats infused with [^{3}H–6]–glucose and either NaH^{14}CO$_3$ or [^{14}C–U]–lactate.

White Gastrocnemius Glycogen (Figure 2.4)

Glycogen turnover in white gastrocnemius muscle was very low at rest as evidenced by the very small amount of ^{14}C incorporation into glycogen at rest (upper panels) and by the almost undectable amount of glycogen synthesized by the direct route (lower panels). Total glycogen was significantly depleted following exercise (from 5.81 $\pm$ 0.60 to 2.93 $\pm$ 0.35 mg/g, $P < 0.05$) (lower left panel), and although it was significantly increased following postexercise recovery (to 4.38 $\pm$ 0.40 mg/g, $P < 0.05$), it remained significantly lower than total glycogen concentration in rested animals ($P < 0.05$) (lower middle panel). Total glycogen synthesis as indexed by the total amount of ^{14}C-glycogen (upper panels) was not statistically significantly different immediately following exercise as compared to rest but was significantly increased during postexercise recovery in both $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats ($P <$ 0.05, compared to rested controls). This same pattern occurred for the amount of glycogen synthesized by the direct uptake of plasma glucose (lower panels). Although there was a statistically significant difference between total and direct ^{14}C-glycogen following recovery in [^{14}C-U]-lactate-infused exercised rats, dividing by the specific activity of plasma lactate gives a very small amount of glycogen made by the glyconeogenic route in the muscle (0.06 $\pm$ 0.01 mg/g) as compared to that made from plasma glucose (1.15 $\pm$ 0.14 mg/g). However minute, this index of gluconeogenic glycogen synthesis was statistically significantly larger than that in rested control animals ($P < 0.05$).

Figure 2.5: Red gastrocnemius glycogen in rested and exercised rats infused with [^{3}H-6]-glucose and either $NaH^{14}CO_3$ or [^{14}C-U]-lactate.
Red gastrocnemius glycogen in rats infused with $NaH^{14}CO_3$ and [^{3}H-6]-glucose was assessed immediately following 4 hours of exercise or rest (no recovery, far left panels) and after 3 hours of recovery (recovery, middle panels). Red gastrocnemius glycogen in [^{14}C-U]-lactate-, [^{3}H-6]-glucose-infused rats was assessed after 4 hours of exercise or rest and 3 hours of recovery (recovery, far right panels). Upper panels: open bars are total ^{14}C-glycogen d.p.m. in red gastrocnemius; striped bars are ^{14}C-glycogen d.p.m. in red gastrocnemius accounted for by the direct uptake of ^{14}C-glucose from plasma. Lower panels: open bars are total red gastrocnemius glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis (negative values do not appear on the graph). All data are expressed as means $\pm$ SEM. **significantly different from total ^{14}C-glycogen, *significantly different from rest, $^+$significantly different from no recovery ($P < 0.05$). $n = 7$ for all bars except those of rested rats with recovery where $n = 6$.

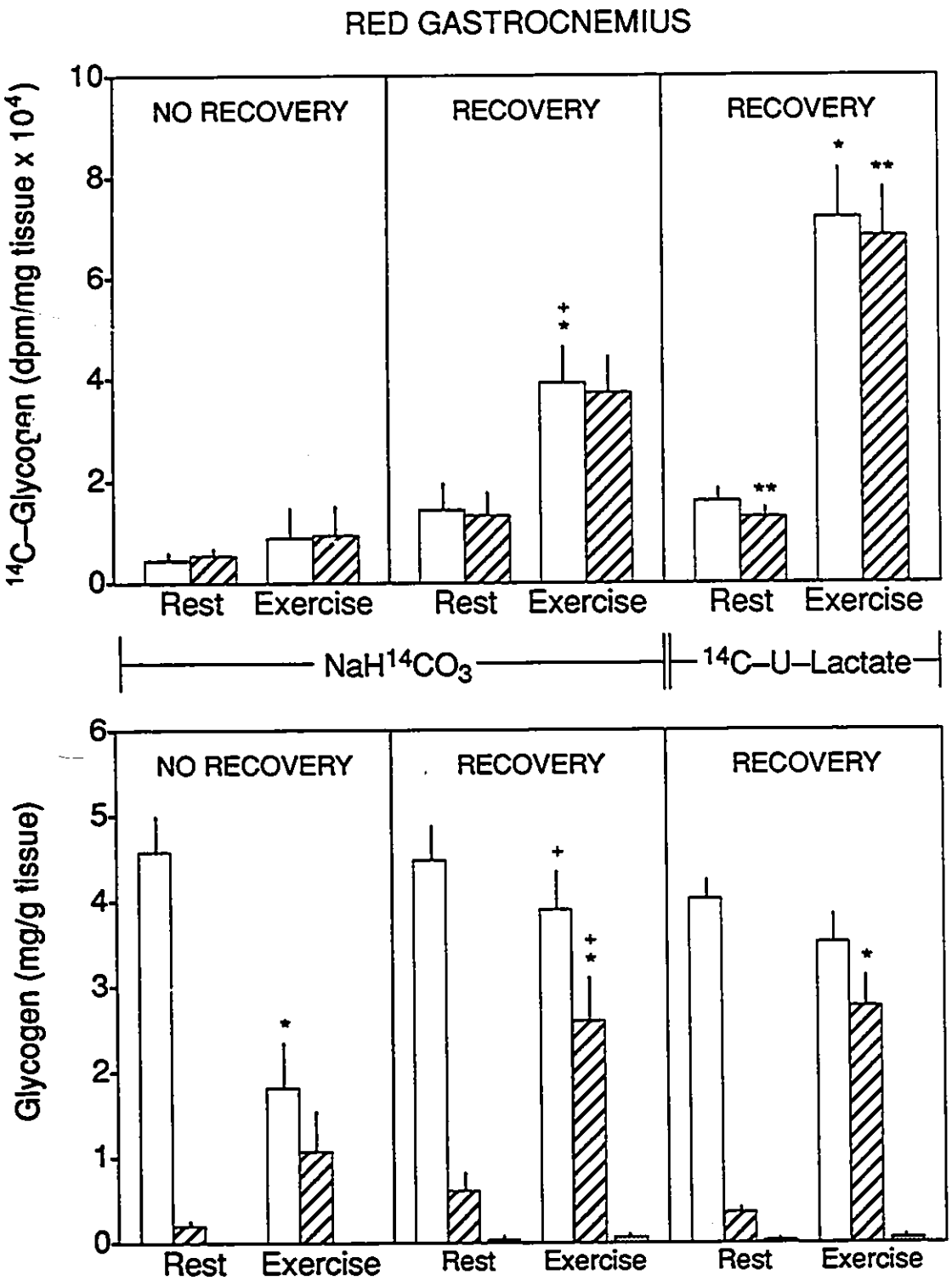

Figure 2.5: Red gastrocnemius glycogen in rested and exercised rats infused with [^{3}H–6]–glucose and either NaH$^{14}CO_3$ or [^{14}C–U]–lactate.

Red Gastrocnemius Glycogen (Figure 2.5)

As in the white gastrocnemius, there was low turnover of glycogen at rest in the red gastrocnemius muscle as depicted by the relatively low ^{14}C-d.p.m. in muscle glycogen (upper panels). Although exercise depleted total glycogen from 4.58 $\pm$ 0.43 to 1.82 $\pm$ 0.51 mg/g ($P < 0.05$) (lower left panel), glycogen was significantly repleted in exercised rats following recovery (3.90 $\pm$ 0.44 mg/g, $P < 0.05$) (lower middle panel). Total ^{14}C-glycogen following postexercise recovery was also significantly higher than that immediately postexercise and in rested control muscle ($P < 0.05$) (upper middle panel). Postexercise ^{14}C incorporation into glycogen in [^{14}C-U]-lactate-infused rats was also significantly higher than in rested controls ($P < 0.05$) (upper right panel). Glycogen synthesis from glucose immediately following four hours of swimming was not statistically significantly higher than that in rested animals (lower left panel). Following three hours of postexercise recovery, however, direct glycogen synthesis was significantly higher as compared to that of rested controls as well as that immediately following exercise ($P < 0.05$) (lower middle panel). Although there were statistically significant differences between total and direct ^{14}C-glycogen in both rested and exercised [^{14}C-U]-lactate-infused animals, the corresponding indices of glycogen made by the glyconeogenic route were nominal (0.03 $\pm$ 0.01 and 0.06 $\pm$ 0.01 mg/g in rested and exercised rats, respectively).

DISCUSSION

METHODOLOGY

Theoretically, all of the ^{14}C-d.p.m. in glycogen in muscle should be accounted for by the direct uptake of ^{14}C-glucose from plasma in NaH$^{14}CO_3$-infused rats as the ^{14}C label from $^{14}CO_2$ would not label the gluconeogenic pathway in muscle. If the glyconeogenic route occurs by the reversal of pyruvate kinase as previously suggested (14, 38), then pyruvate would be phosphorylated directly to phosphoenolpyruvate (PEP), without condensing with $^{14}CO_2$, thus not incorporating the label into the pathway at all. If, on the other hand, muscle glyconeogenesis involves the carboxylation of pyruvate via malic enzyme (13, 41), the $^{14}CO_2$ label would be incorporated at this first step but lost in the subsequent route to PEP. This is due to the incomplete equilibration in the fumarase reaction in muscle (7) which fails to randomize the ^{14}C label on oxaloacetate (OAA) between the first and fourth carbons via formation of the symmetrical fumarate molecule. As a result, the ^{14}C label on carbon 1 of OAA will be lost as $^{14}CO_2$ in the formation of PEP. The observation that total and direct ^{14}C-glycogen in soleus, white and red gastrocnemii are indeed equal (within reasonable experimental error) (Figures 2.3, 2.4, 2.5) supports the theoretical expectations and justifies the use of these differences as the basis for the statistical minimal detectable difference test used in [^{14}C-U]-lactate-infused rats.

The small differences that do occur between total and direct ^{14}C-glycogen in the muscles may be due to the limitations of the tracer methodology (for example, measurements of changing plasma ^{3}H/^{14}C with time) and the nature of the compari-

sons. Total ^{14}C-glycogen is just that, one (mean) measurement of the total amount of ^{14}C-d.p.m. in glycogen; direct ^{14}C-glycogen, on the other hand, is the mean result of a calculation of seven different (mean) measurements, each with its own error. Another possible reason for the slight discrepancies between total and direct ^{14}C-glycogen in the muscles could be due, again, to the tracer methods chosen. $^{14}CO_2$ could label glucose in the 3,4 positions through the gluconeogenic pathway in the liver. This labelled glucose could enter the plasma, be taken up by the muscle, oxidized to ^{14}C-lactate which could then form glycogen gluconeogenically within the muscle, labelling the glycogen pool with ^{14}C. The number of reactions and opportunities for both dilution and/or losses of the label, however, make this consideration a minor one, especially in the light of the observations that total and direct ^{14}C-glycogen are experimentally virtually equal.

LIVER

The liver was included in this study initially as a means of validating the tracer methods used to detect glyconeogenesis as it is well documented that at least half of hepatic glycogen synthesis during refeeding is via the indirect or glyconeogenic route (eg. 24, 25, 26, 29, 30, 31, 34, 35, 37). Indeed, our studies confirm that a substantial amount of hepatic glycogen is made by the gluconeogenic route at rest, during exercise and during postexercise recovery in the fasted state. The indices of glyconeogenesis, however, are only minimal estimates of the amount of glycogen synthesized by this route as they have not been corrected for the metabolic exchange

of ^{14}C for ^{12}C in the Krebs cycle. If the correction factor is close to 2 (21, 34) using both $^{14}CO_2$ and ^{14}C-lactate as tracers, then gluconeogenic precursors are the predominant substrate for hepatic glycogen synthesis, at least during a fasted postexercise recovery period.

The data reveals the patterns of liver glycogen depletion and repletion at rest, during exercise and during postexercise recovery. It is our experience, as well as others (44), that hepatic glycogen concentrations in fasted rested animals can vary considerably (up to 2- and 3-fold) depending on procedures and feeding schedules in the animal facilities and may not reflect true experimental differences. Quantititative comparisions with these resting control values, then, are made with less certainty. Exercised rats with or without recovery periods, on the other hand, have shown reproducible hepatic glycogen concentrations. Exercise not only depleted liver glycogen to very low levels (as discussed in Chapter 1) but also decreased the amounts of glycogen made by both the direct and indirect routes.

MUSCLE GLYCOGEN SYNTHESIS

The results of this study demonstrate that *1)* at rest, the rates of glycogen synthesis are highest in soleus (SO fibers) followed by the red (FOG) and white (FG) gastrocnemii, respectively, *2)* during exercise, new glycogen synthesis occurred despite net glycogen depletion in the fast-twitch muscles, *3)* prior exercise stimulated postexercise (direct) glycogenesis in white and red gastrocnemii, while having little effect on direct synthesis in the soleus, and *4)* there was little indication of any

gluconeogenic synthesis of muscle glycogen.

Hutber and Bonen (23) have reported that at rest and during submaximal running, the order of rates of net glycogen synthesis was SO > FOG > FG which is in agreement with our findings. Their reports of 189% and 400% increases in rates of direct glycogen synthesis in red and white gastrocnemii, respectively, after 90 minutes of submaximal running (23) are similar to the 162% (red gastrocnemius) and 310% (white gastrocnemius) increases following four hours of swimming in the present study. (These changes were calculated as the percent increases in ^{14}C-d.p.m. in glycogen from the uptake of ^{14}C-glucose in plasma immediately following the swim period in rested *versus* exercised rats.) The amount of glycogen synthesized from glucose in the soleus muscle did not change from rest to exercise. This is in agreement with the study of Hutber and Bonen (23) which showed initial decreases but finally no net changes in the rates of glucose incorporation into glycogen in the soleus after 90 minutes of running as compared to that at rest. The amounts of glycogen synthesized by the direct routes during exercise were similar in soleus (1.18 mg/g) and red gastrocnemius (1.07 mg/g), which were slightly higher than that in the white muscle (0.89 mg/g).

SOLEUS MUSCLE

Compared to white and red gastrocnemii, the soleus muscle showed relatively lower total glycogen content as well as very high turnover of glycogen at rest, with approximately 40% replacement. This is in keeping with the nature of the large

proportion of slow oxidative fibers in this muscle (72%: Hutber and Bonen (23); 84%: Ariano et al. (2); 87%: Armstrong and Phelps (3)). PAS (periodic acid-Schiff) staining of muscle fibers in the plantaris at rest have shown much higher concentrations of glycogen in both fast oxidative glycolytic fibers (FOG) and fast glycolytic fibers (FG) compared to slow oxidative fibers (SO) (4). In the recruitment pattern of muscle fiber activation, SO fibers are activated first as they require low force to stimulate contraction. When the rat is simply standing, blood flow is directed primarily to the extensor, antigravity muscle groups which are composed of slow-twitch fibers (4). Thus, these muscles are being activated continuously, requiring energy and the repletion of energy. This would account for the relatively higher rates of glycogen synthesis without net glycogen deposition.

The soleus muscle was not depleted to any significant degree following four hours of swimming. This contradicts previous reports of significant glycogen depletion in the soleus muscle following prolonged swimming (12, 15, 39, 42, 43). The discrepancies are most likely due to the nature of the exercises involved. In all of these previous studies, several rats swam simultaneously and "competetively" (in a barrel or tank), constantly interacting with one another, pushing off each other as well as off the sides and bottom of the tanks - activities which would recruit the soleus muscle to repeatedly extend the hindfeet. In our swimming rat model, each rat swam alone in a segregated area and could not access the sides or bottom of the tank due to the restrictions imposed by the tether systems. Thus, the positions of the hindfeet remained more or less extended during the swimming strokes and the soleus would

have been required for neither antigravity functions (due to the buoyancy in the water) nor for repeated extensions of the hindfeet.

Another possible explanation for the lack of glycogen depletion in the soleus is that, when recruited, the muscle was oxidizing fuel sources other than muscle glycogen, such as plasma free fatty acids and/or intramuscular lipids. Significant decreases (50%) in intramuscular triacylglycerol content have been reported in soleus muscle in rats following prolonged exhaustive swimming, while the swim had no effect on intramuscular lipid content in the white gastrocnemius (39). The "competitive" and perhaps more stressful swims in the other studies may have elicited hormonal profiles more conducive to the breakdown of glycogen rather than to the oxidation of fat (intramuscular sources or plasma free fatty acids) for fuel. As another alternative fuel source to muscle glycogen, the slow-twitch oxidative fibers of the soleus may have been oxidizing lactate produced in the fast-twitch muscle fibers. In his "lactate shuttle" hypothesis, Brooks (10, 11) suggests that during sustained submaximal exercise, lactate is released from contracting muscle fibers and taken up and combusted by other muscle fibers with high respiratory capacities. These are some possible explanations, then, to account for the lack of significant glycogen depletion in the soleus muscles after the prolonged swim.

During postexercise recovery, however, there was a net decrease in soleus glycogen. A previous study reported no increase in glycogen in the soleus muscle following three hours of a fasted recovery from exhaustive swimming in rats, but significant decreases in soleus glycogen with propranolol (β-blockade), nicotinic acid

(inhibition of lipolysis) and dichloracetate (enhances pyruvate oxidation) treatments (15). It is tempting to suggest that glucose uptake by the soleus is decreased during the postexercise recovery period in favour of preferential glycogen repletion in the fast-twitch muscles which rely more heavily on glycogen as a fuel source. Yet, this hypothesis is not consistent with the observation that the amount of glycogen synthesized by the direct uptake of plasma glucose did not decrease during or following exercise as compared to resting values. The only possible explanation for the net decrease in soleus glycogen during postexercise recovery is that the rate of glycogenolysis exceeded the rate of glycogenesis. Indeed, during postexercise recovery, the rats were replaced in their cages and, therefore, would once again stimulate contractions of slow oxidative antigravity muscles and thus glycogenolysis in the soleus muscle. This increased breakdown of the glycogen pool in the soleus (due to whatever stimulus) would not only supply local energy to the muscle itself but could also, theoretically, provide substrate for the repletion of glycogen in the depleted muscles. Since muscles do not have the capability of releasing glucose into the plasma directly, glucose units in the soleus would have to be oxidized to lactate and exit the cells in the form of lactate or alanine. This would allow fast-twitch muscles to take up lactate from adjacent muscles (via the circulation) or, more likely, the resulting glucose from hepatic gluconeogenesis from this lactate. Ahlborg and Felig (1) have previously suggested redistribution of muscle glycogen following prolonged submaximal exercise whereby the previously inactive muscle (forearm) released lactate at rates in excess of glucose uptake, while exercised muscle (leg) took up

glucose at rates three to five times those of basal. In this way, the nonexercised or glycogen-rich muscle (soleus) may provide the substrate for hepatic gluconeogenesis for net production of glycogen in the exercised, glycogen-depleted muscles (gastrocnemii).

All glycogen in the soleus muscle was synthesized from plasma glucose. There was no evidence of any gluconeogenic glycogen formation in the muscle as traced using [^{14}C-U]-lactate. This is in keeping with both theoretical expectations and previous findings. McLane and Holloszy (28) have shown very little ^{14}C-lactate incorporation into soleus glycogen in the perfused hindlimb (with and without high levels of lactate, glucose or insulin in the perfusion medium), apparently due to extremely low levels fructose-1,6-bisphosphatase activity and relatively low PEPCK activity, two key gluconeogenic enzymes. Rats recovering from exhaustive treadmill running also showed no gluconeogenic repletion of soleus glycogen as traced by ^{14}C-lactate infusion with and without high lactate or glucose levels (24). The apparent inability of the soleus muscle to synthesize glycogen from lactate, again, may provide fast-twitch muscle fibers with substrate (either via glyconeogenesis or direct uptake of plasma glucose resulting from hepatic gluconeogenesis from this lactate) during times of depletion for the purpose of glycogen repletion, since glycogen utilization is not as critical to the soleus muscle in a "fight or flight" situation as it is to the fast-twitch fibers.

WHITE AND RED GASTROCNEMII

In as much as the soleus exhibited high glycogen turnover at rest, the fast-twitch red and white fibers of the gastrocnemius muscles demonstrated very low glycogen turnover at rest. This again is consistent with the recruitment of muscle fibers. Fast-twitch white fibers are recruited last as they require more force to stimulate contraction and are used with increasing levels of force in higher intensity work or when other fibers become depleted of glycogen (3, 4). Following the prolonged swim, however, glycogen was depleted 50% in white muscle and 60% in the red gastrocnemii, respectively, as detected in Series I rats. Previous reports have also found that fast-twitch red muscles are depleted to the greatest degree in prolonged low intensity exercise (4, 13, 43) as well as having the highest postexercise rate of glycogen repletion (13, 43).

Although McLane and Holloszy (28) have demonstrated not only significant activities of enzymes in a potential glyconeogenic pathway, but also the direct incorporation of ^{14}C-lactate into glycogen in perfused white and red gastrocnemii, glycogen synthesis in the gastrocnemius muscles following prolonged swimming in the present study seems to have been almost entirely from the direct uptake of plasma glucose as no evidence of any significant amounts of gluconeogenic glycogen formation was detected as traced by [^{14}C-U]-lactate infusion into plasma. These results could be interpreted in one of two ways: firstly, that muscle glycogen is repleted primarily via plasma glucose. This interpretation would agree with some previous reports, that, during a fasted recovery period, the major postexercise fate of

lactate is oxidation and that conversion of lactate to muscle glycogen occurs primarily via hepatic gluconeogenesis (9, 17, 33). Johnson and Bagby (24) have reported that white gastrocnemius is capable of significant gluconeogenic glycogen repletion but only in the presence of high lactate levels produced by nutritive support. In our swimming rat model, the postexercise lactate concentration was only marginally above basal (1.3 mM), and thus plasma would probably not be a major source of the glyconeogenic substrate for muscle.

The second possible interpretation of the results would be that infusion of [^{14}C-U]-lactate is not the most efficacious means of detecting muscle glyconeogenesis, especially in light of the fact that plasma lactate does not appear to be a major contributor to glycogen repletion except under conditions of high lactataemia (24, 39). Infusion of [^{14}C-U]-lactate into plasma would only trace glycogen synthesis from plasma lactate and may not adequately label intramuscular or locally formed lactate. It may be, then, that muscle glycogen is repleted via glyconeogenesis from lactate formed locally in the muscle which equilibrates minimally with plasma lactate and, therefore, gluconeogenic formation of glycogen from this source may go undetected.

REFERENCES

1. Ahlborg, G., and P. Felig. Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. J. Clin. Invest. 69: 45-54, 1982.

2. Ariano, M. A., R. B. Armstrong, and V. R. Edgerton. Hindlimb muscle fiber populations of five mammals. J. Histochem. Cytochem. 21(1): 51-55, 1973.

3. Armstrong, R. B., and R. O. Phelps. Muscle fiber type composition of the rat hindlimb. Am. J. Anat. 171: 259-272, 1984.

4. Armstrong, R. B., C. W. Saubert, W. L. Sembrowich, R. E. Shepard, and P. D. Gollnick. Glycogen depletion in rat skeletal muscle fibers at different intensities and durations of exercise. Pflügers Arch. 352: 243-256, 1974.

5. Åstrand, P. -O., E. Hultman, A. Juhlin-Dannfelt, and G. Reynolds. Disposal of lactate during and after strenuous exercise in humans. J. Appl. Physiol. 61(1): 338-343, 1986.

6. Bendall, J. R., and A. A. Taylor. The Meyerhof quotient and the synthesis of glycogen from lactate in frog and rabbit muscle. Biochem. J. 118: 887-803, 1970.

7. Bloom, B., and W. Foster. Source of phosphoenolpyruvate for hexose synthesis in liver and muscle as studied with aspartate-3-C^{14}. J. Biol. Chem. 237(9): 2744-2746, 1962.

8. Bonen, A., J. C. McDermott, and M. H. Tan. Glycogenesis and glyconeogenesis in skeletal muscle: effects of pH and hormones. Am. J. Physiol. 258 (Endocrinol. Metab. 21): E693-E700, 1990.

9. Brooks, G. A., and G. A. Gaesser. End points of lactate and glucose metabolism after exhausting exercise. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(6): 1057-1069, 1980.

10. Brooks, G. A. Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. Fed. Proc. 45: 2924-2929, 1986.

11. Brooks, G. A. The lactate shuttle during exercise and recovery. Med. Sci. Sports Exerc. 18(3): 360-368, 1986.

12. Conlee, R. K., R. C. Hickson, W. W. Winder, J. M. Hagberg, and J. O. Holloszy. Regulation of glycogen resynthesis in muscles of rats following exercise. Am. J. Physiol. 235 (Regulatory Integrative Comp. Physiol. 4): R145-R150, 1978.

13. Connett, R. J. Glyconeogenesis from lactate in frog striated muscle. Am J. Physiol. 237(5): C231-C236, 1979.

14. Dyson, R. D., J. M. Cardenas, and R. J. Barsotti. The reversibility of skeletal muscle pyruvate kinase and an assessment of its capacity to support glyconeogenesis. J. Biol. Chem. 250(9): 3316-3321, 1975.

15. Favier, R. J., H. E. Koubi, M. H. Mayet, B. Semporé, B. Simi, and R. Flandrois. Effects of gluconeogenic precursor flux alterations on glycogen resynthesis after prolonged exercise. J. Appl. Physiol. 63(5): 1733-1738, 1987.

16. Fell, R. D., J. A. McLane, W. W. Winder, and J. O. Holloszy. Preferential resynthesis of muscle glycogen in fasting rats after exhausting exercise. Am. J. Physiol. 238 (Regulatory Integrative Comp. Physiol. 7): R238-R332, 1980.

17. Gaesser, G. A., and G. A. Brooks. Glycogen repletion following continuous and intermittent exercise to exhaustion. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(4): 722-728, 1980.

18. Gleeson, T. Glycogen synthesis from lactate in skeletal muscle of the lizard *Dipsosaurus dorsalis*. J. Comp. Physiol. B. 156: 277-283, 1985.

19. Goodman, M. N., R. Dietrich, and P. Luu. Formation of gluconeogenic precursors in rat skeletal muscle during fasted-refed transition. Am. J. Physiol. 259 (Endocrinol. Metab. 22): E513-E516, 1990.

20. Hermansen, L., and O. Vaage. Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. Am J. Physiol. 233(5): E422-E429, 1977.

21. Hetenyi, G., Jr. Correction for the metabolic exchange of ^{14}C for ^{12}C atoms in the pathway of gluconeogenesis *in vivo*. Fed. Proc. 41: 104-109, 1982.

22. Hiatt, H. H., M. Goldstein, J. Laureau, and B. L. Horecker. The pathway of hexose synthesis from pyruvate in muscle. J. Biol. Chem. 231: 303-307, 1958.

23. Hutber, C. A., and A. Bonen. Glycogenesis in muscle and liver during exercise. J. Appl. Physiol. 66(6): 2811-2817, 1989.

24. Johnson, J. L., and G. J. Bagby. Gluconeogenic pathway in liver and muscle glycogen synthesis after exercise. J. Appl. Physiol. 64(4): 1591-1599, 1988.

25. Katz, J., and J. D. McGarry. The glucose paradox: is glucose a substrate for liver metabolism? J. Clin. Invest. 74: 1901-1909, 1984.

26. Lang, C. H., G. J. Bagby, H. L. Blakesley, J. L. Johnson, and J. J. Spitzer. Plasma glucose concentration determines direct *versus* indirect liver glycogen synthesis. Am. J. Physiol. 251 (Endocrinol. Metab. 14): E584-E590, 1986.

27. Maehlum, S., P. Felig, and J. Wahren. Splanchnic glucose and muscle glycogen metabolism after glucose feeding during postexercise recovery. Am. J. Physiol. 235(3): E255-E260, 1978.

28. McLane, J. A., and J. O. Holloszy. Glycogen synthesis from lactate in the three types of skeletal muscle. J. Biol. Chem. 254: 6548-6553, 1979.

29. Mitrakou, A., R. Jones, Y. Okuda, J. Pena, N. Nurjhan, J. B. Field, and J. E. Gerich. Pathway and carbon sources for hepatic glycogen repletion in dogs. Am. J. Physiol. 260 (Endocrinol. Metab. 23): E194-E202, 1991.

30. Newgard, C. B., S. V. Moore, D. W. Foster, and J. D. McGarry. Efficient hepatic glycogen synthesis in refeeding rats requires continued carbon flow through the gluconeogenic pathway. J. Biol. Chem. 259: 6958-6963, 1984.

31. Newgard, C. B., L. J. Hirsch, D. W. Foster, and J. D. McGarry. Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat. J. Biol. Chem. 258: 8046-8052, 1983.

32. Pagliassotti, M. J., C. M. Donovan. Glycogenesis from lactate in rabbit skeletal muscle fiber types. Am. J. Physiol. 258 (Regulatory Integrative Comp. Physiol. 27): R903-R911, 1990.

33. Peters Futre, E. M., T. D. Noakes, P. I. Raine, and S. E. Terblanche. Muscle glycogen repletion during active postexercise recovery. Am. J. Physiol. 253 (Endocrinol. Metab. 16): E305-E311, 1987.

34. Radziuk, J. Sources of carbon in hepatic glycogen synthesis during absorption of an oral glucose load in humans. Fed. Proc. 41:110-116, 1982.

35. Radziuk, J. Tracer methods and the metabolic disposal of a carbohydrate load in man. Diabetes/Metabolism Reviews 3(1): 231-267, 1987.

36. Rodbard, D. Statistical estimation of the minimal detectable concentration ("sensitivity") for radioligand assays. Anal. Biochem. 90: 1-12, 1978.

37. Scofield, R. F., L. Kosugi, W. C. Shumann, K. Kumaran, and B. R. Landau. Quantitative estimation of the pathways followed in the conversion to glycogen of glucose administered to the fasted rat. J. Biol. Chem. 260: 8777-8782, 1985.

38. Shiota, M., S. Golden, and J. Katz. Lactate metabolism in the perfused rat hindlimb. Biochem. J. 222: 281-292, 1984.

39. Spriet, L. L., S. J. Peters, G. J. F. Heigenhauser, and N. L. Jones. Rat skeletal muscle triacylglycerol utilization during exhaustive swimming. Can. J. Physiol. Pharmacol. 63: 614-618, 1985.

39. Stevenson, R. W., D. R. Mitchell, G. K. Hendrick, R. Rainey, A. D. Cherrington, and R. T. Frizzell. Lactate as substrate for glycogen resynthesis after exercise. J. Appl. Physiol. 62(6): 2237-2240, 1987.

40. Talmadge, R. J., J. I. Scheide, and H. Silverman. Glycogen synthesis from lactate in chronically active muscle. J. Appl. Physiol. 66(5): 2231-2238, 1989.

41. Talmadge, R. J., and H. Silverman. Glyconeogenic and glycogenic enzymes in chronically active and normal skeletal muscle. J. Appl. Physiol. 71(1): 182-191, 1991.

42. Terblanche, S. E., R. D. Fell, A. C. Juhlin-Dannfelt, B. W. Craig, and J. O. Holloszy. Effects of glycerol feeding before and after exhausting exercise in rats. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 50(1): 94-101, 1981.

43. Terjung, R. L., K. M. Baldwin, W. W. Winder, and J. O. Holloszy. Glycogen repletion in different types of muscle and in liver after exhausting exercise. Am. J. Physiol. 226(6): 1387-1391, 1974.

44. Vissing, J., B. Sonne, and H. Galbo. Role of metabolic feedback regulation in glucose production of running rats. Am. J. Physiol. 255 (Regulatory Integrative Comp. Physiol. 24): R400-R406, 1988.

CHAPTER 3: INTRACELLULAR GLUCOSE RECYCLING AS AN INDEX OF MUSCLE AND LIVER GLYCONEOGENESIS

INTRODUCTION

The results from Chapter 2 suggest that prolonged swimming induces increased rates of postexercise glycogen synthesis in white and red gastrocnemii but to a lesser degree in liver, and not at all in soleus. Glycogen synthesis in the soleus and white and red gastrocnemius muscles appeared to be virtually all from the direct uptake of plasma glucose; yet, there was evidence of some glyconeogenic formation in the white and red gastrocnemii, albeit at seemingly extremely low rates as traced by ^{14}C-lactate.

Because equilibration with tissue lactate may be incomplete, however, ^{14}C-lactate infusion into the plasma may only track glyconeogenesis from systemic lactate. We hypothesized that perhaps glycogen could be synthesized from lactate which is formed locally in the muscles and never enters the general circulation; therefore, glyconeogenesis from this source may not be adequately labelled using ^{14}C-lactate infusion into plasma. Support for this hypothesis comes from the following three sources. Firstly, it has been suggested that there is a "lactate shuttle" by which lactate formed in active muscle fibers may be transported (via diffusion, through the interstitium or vasculature) into other adjacent muscle fibers for oxidation (5). Oxidation, however, need not be the only postexercise fate of this local lactate; theoretically, it could be used to synthesize glycogen. Secondly, in Chapter 2, we noted net depletion of soleus glycogen during postexercise recovery, without a

decrease in the amount of glycogen synthesized from glucose. This may suggest that perhaps lactate formed from soleus glycogen during recovery (which is not expected to be high since the muscle has high respiratory capacity) was being transported into adjacent muscle fibers for glyconeogenesis without mixing with the systemic lactate pool. Finally, Talmadge et al. (33) have not only demonstrated ^{14}C-lactate incorporation into nonactive superficial fast-twitch fibers of the chronically active gastrocnemius muscle in C57B1/6J dy^{2J}/dy^{2J} mice, but have also attributed the increased glycogen stores in these muscle fibers to glyconeogenesis from the high levels of lactate formed locally in the muscle in the chronically active fibers.

In an attempt to label intramuscularly formed lactate more effectively and to trace any glyconeogenic activity from this source, [^{14}C-1]-glucose was infused into rats during and following prolonged swimming or rest. ^{14}C label redistributed in the glucosyl units of tissue glycogen not accounted for by direct uptake of recycled ^{14}C-glucose from plasma was used as an index of gluconeogenic glycogen formation in muscle (or liver) immediately following the prolonged swim and after three hours of postexercise recovery. (Refer to Introduction (p. 35) for explanation of "recycled" ^{14}C-glucose.)

METHODS

EXPERIMENTAL PROTOCOL

The same experimental protocol as described in Chapters 1 and 2 was used except that the rats were infused with [^{14}C-1]-glucose and D-[^{3}H-3]-glucose (or D-[^{3}H-6]-glucose in a few rats only). The priming dose for [^{14}C-1]-glucose was approximately 3.5 μCi which was followed by a constant intravenous infusion of approximately 0.03 μCi/min; tritiated glucose was infused at rates previously described. Rats were sacrificed immediately following the four-hour swim, along with their rested controls. A second group of rats (exercised and nonexercised) was sacrificed following a three-hour postexercise recovery period. Blood and tissue samples were taken and processed as described in Chapters 1 and 2.

CHEMICAL ASSAYS

Plasma samples were deproteinized and run through the columns as described in Chapter 1. Recycled glucose in plasma was determined as described below. Total and radiolabelled liver and muscle glycogen was determined as described in Chapter 2. In order to determine ^{14}C-d.p.m. in tissue glycogen arising from recycled ^{14}C-glucose, 0.5 ml of the hydrolyzed glycogen solutions were first neutralized with 1 N NaOH and made up to a final volume of 2.5 ml before undergoing the glucose recycling assay.

Glucose recycling assay. The quantitation of the activity of ^{14}C in the C-6 position of glucose was achieved using the method of Reeves (28). In the presence of periodic acid, the terminal carbinol group (C-6 position) is cleaved off as formaldehyde which can then be precipitated as a measurable product. To 1 ml of a standard glucose solution (1 g/100 ml) and 3 ml of the first column eluate sample (containing glucose) (or 2 ml of neutralized muscle or liver glycogen solution) was added 2 ml of 7% periodic acid and 2 ml of 1 M sodium bicarbonate. The samples were mixed and allowed to stand at room temperature for one hour. Upon addition of 1 N HCl (3 ml) and 1.2 N sodium arsenite (2 ml), a brown precipitate formed but disappeared upon swirling the flasks. Sodium acetate (1 N, 2 ml) was added along with 1 ml of 8% (w/v in ethanol) dimedone (5,5-dimethyl 1,3-cyclohexanedione). Precipitation of the product (formaldehyde-dimedone) was complete after heating for 10 minutes at 100°C. The white precipitate was collected on Whatman #5 filter paper, allowed to air dry, weighed and counted in scintillation fluid. [^{14}C-1]-glucose as well as [^{14}C-6]- and [^{3}H-6]-glucose standards were run through the assay in parallel with the samples so as to determine the correction factor (see below) and the recoveries of label in the C-6 position, respectively.

Recycled glucose was determined as

$$recycled\ [^{14}C]\text{-}glucose = [^{14}C\text{-}6]\text{-}glucose \times 5 \qquad (3.1)$$

whereby [^{14}C-6]-glucose ([^{14}C-6]-G) was calculated by

$$[^{14}C\text{-}6]\text{-}G = \left[\frac{wafer\ [^{14}C]\text{-}d.p.m. \times T.W.}{A.W.} \times dilution\right] - correction \qquad (3.2)$$

where *dilution* is the dilution factor for the sample, *A.W.* is the actual wafer weight, *T.W.* is the theoretical weight of the product wafer and *correction* is the correction factor for ^{14}C-d.p.m. arising from [^{14}C-6]-glucose in the [^{14}C-1]-glucose tracer. ([^{3}H-6]-glucose was calculated in the same way without the correction factor.) T.W. was calculated by

$$T.W. = (glucose\ mg/ml \times dilution \times 1.622) + 16.22 \qquad (3.3)$$

where *dilution* is the dilution factor of the sample and 16.22 is the amount of product (mg) made from 10 mg glucose (i.e. amount of carrier glucose). The correction factor was determined by

$$correction = \left[\frac{\frac{wafer\ [^{14}C]\text{-}d.p.m. \times T.W.}{A.W.}}{Volume \times total\ [^{14}C]\text{-}d.p.m.}\right] \times \frac{[^{14}C]\text{-}d.p.m.}{ml} \qquad (3.4)$$

where the *wafer* ^{14}*C-d.p.m.*, *T.W.* (theoretical wafer weight) and *A.W.* (actual wafer weight) are those of the [^{14}C-1]-glucose (and tritiated glucose) infusate standard (1:100 dilution of tracer solution). *Volume* is the amount (ml) of infusate standard used in the recycling assay, *total* ^{14}*C-d.p.m.* are the those in the infusate standard and ^{14}*C-d.p.m./ml* are those of the plasma or glycogen sample.

CALCULATIONS

Glycogen synthesized by the direct pathway was calculated as in Chapter 2. ^{14}C-glycogen made from the direct uptake of recycled ^{14}C-glucose was calculated by

$$direct\ [^{14}C]\text{-}glycogen = \frac{[^{3}H]\text{-}glycogen\ d.p.m.}{\frac{[^{3}H]\text{-}glucose\ d.p.m.}{recycled\ [^{14}C]\text{-}glucose\ d.p.m.}} \qquad (3.5)$$

where *^{3}H-glycogen d.p.m.* are those in tissue glycogen, and labelled glucose d.p.m. are those in plasma averaged during the exercise or recovery period. An index of glyconeogenesis was estimated by

$$index = \frac{total\ [^{14}C]\text{-}glycogen\ -\ direct\ [^{14}C]\text{-}glycogen}{SA\ plasma\ [^{14}C]\text{-}lactate} \qquad (3.6)$$

where *total ^{14}C-glycogen* is the total amount of recycled ^{14}C-glucose in glycogen, *direct ^{14}C-glycogen* is that calculated from equation 3.5 and *SA* is the average specific activity of plasma lactate during the exercise or recovery period. (It was necessary to use the specific activity of plasma rather than muscle lactate as the latter was not determined due to the limited muscle tissue resources.)

STATISTICAL ANALYSIS

Significant differences between total recycled ^{14}C-d.p.m. in muscle and liver glycogen and that accountable by the direct uptake of recycled ^{14}C-glucose from plasma were detected using the minimal detectable difference test as described in Chapter 2. The ratios of ^{3}H-glucose to ^{14}C-glucose in $NaH^{14}CO_3$-infused rats were

comparable to the ratios of ^{3}H- to recycled ^{14}C-glucose in [^{14}C-1]-glucose-infused animals, justifying the use of the ^{14}C-d.p.m. minimal detectable differences calculated using $NaH^{14}CO_3$-infused rats. All other statistical analyses were done using analysis of variance with a completely randomized factorial design and an *a posteriori* comparison by Scheffe's procedure on a SAS P.C. package; the level of significance was set at $P < 0.05$.

RESULTS

Liver Glycogen (Figure 3.1)

Rest. Total fasted hepatic glycogen concentration was 2.25 ± 0.72 mg/g liver in six-hour rested controls (lower left panel). Total liver glycogen in nine-hour rested animals (1.31 ± 0.40 mg/g, lower right panel) was not statistically significantly different as compared to six-hour rested animals. (The large error bars reflect the variability in glycogen concentrations in the rested animals.) Hepatic glycogen formation in the resting fasted state was very low. Glycogen synthesized by the direct route was 0.43 ± 0.23 mg/g in six-hour and 0.26 ± 0.09 mg/g liver in nine-hour rested animals, respectively (lower panels). The observation that recycled ^{14}C-glucose in glycogen arising from the direct uptake of recycled ^{14}C-glucose from plasma was significantly lower than total recycled ^{14}C-glucose in glycogen ($P < 0.05$) is indicative of significant gluconeogenic formation of glycogen in the liver at rest (upper panels). In fact, dividing these differences by the average specific activities of plasma

lactate (during the four-hour rest period or three-hour recovery period), gives uncorrected indices of gluconeogenic glycogen synthesis of 0.29 ± 0.08 mg/g and 0.22 ± 0.06 mg/g liver in rested animals before and after the recovery period, respectively. If the correction factor for these indices is assumed to be approximately 2 (17, 26), then the amounts of glycogen synthesized by the indirect route are almost double the contributions made via the direct route at rest.

Exercise (No Recovery). Exercise depleted total liver glycogen to 0.29 ± 0.03 mg/g, a value not statistically different from the rested condition, most likely due to the large variations in total hepatic glycogen in rested animals (lower left panel). Hepatic glycogen synthesis was not statistically significantly decreased during the prolonged swim as indexed by total recycled ^{14}C-glucose incorporation into glycogen (upper left panel) and direct glycogen synthesis (0.13 ± 0.06 mg/g, lower left panel) (again presumably due to the large standard errors in resting conditions, upper left panel). The significant difference between total and direct incorporation of recycled ^{14}C-glucose into glycogen ($P < 0.05$, upper left panel) translated into an uncorrected glyconeogenic index of 0.12 ± 0.04 mg/g, thus matching the amount of glycogen made via the direct route (lower left panel). Correction for label dilution, however, would make glyconeogenesis the predominant means of glycogen synthesis at double the rate of direct glycogen synthesis in the liver.

Figure 3.1: Liver glycogen in rested and exercised rats infused with [^{3}H-3]- and [^{14}C-1]-glucose.
Liver glycogen in rats infused with [^{3}H-3]- and [^{14}C-1]-glucose was assessed immediately following 4 hours of exercise or rest (no recovery, left panels) and after 3 hours of recovery (3 hours recovery, right panels). Upper panels: open bars are total recycled ^{14}C-glucose d.p.m. in liver glycogen; striped bars are recycled ^{14}C-glucose d.p.m. in liver glycogen accounted for by the direct uptake of recycled ^{14}C-glucose from plasma. Lower panels: open bars are total liver glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis. All data are expressed as means $\pm$ SEM. **significantly different from total recycled ^{14}C-glucose d.p.m. in liver glycogen ($P < 0.05$). $n = 6$ for all bars.

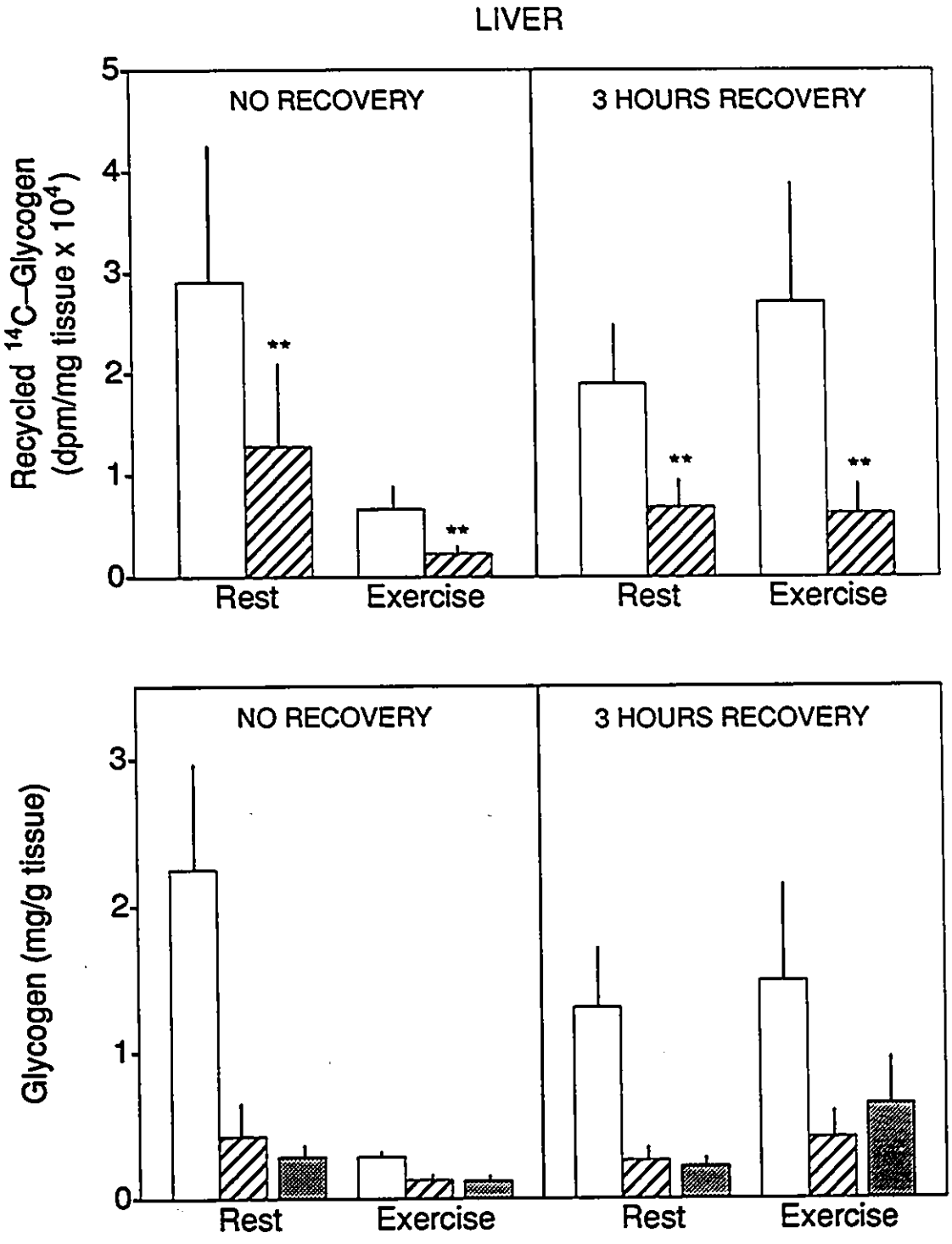

Figure 3.1: Liver glycogen in rested and exercised rats infused with [^{3}H–3]– and [^{14}C–1]–glucose.

Postexercise Recovery. Both total hepatic glycogen (1.49 $\pm$ 0.66 mg/g, lower right panel) and total ^{14}C-glycogen from recycled ^{14}C-glucose (2.71 x 10^4 $\pm$ 1.16 x 10^4 d.p.m./mg liver, upper right panel) during postexercise recovery were not statistically significantly different from those in rested controls nor in exercised animals with no recovery. The amounts of glycogen synthesized by both the direct (0.42 $\pm$ 0.18 mg/g liver, lower right panel) and glyconeogenic routes (0.65 $\pm$ 0.31 mg/g) were not significantly changed after three hours of a fasted postexercise recovery period. The glyconeogenic index is the uncorrected quantitative estimate of gluconeogenic glycogen corresponding to the apparent substantial amount of ^{14}C-glycogen not arising from the direct uptake of recycled ^{14}C-glucose from plasma (upper right panel). Again, as in rest and exercise, a correction of the index of glyconeogenesis during postexercise recovery would reveal the indirect route as the primary pathway of glycogen synthesis in the liver.

Figure 3.2: Soleus glycogen in rested and exercised rats infused with [^{3}H-3]- and [^{14}C-1]-glucose.
Soleus glycogen in rats infused with [^{3}H-3]- and [^{14}C-1]-glucose was assessed immediately following 4 hours of exercise or rest (no recovery, left panels) and after 3 hours of recovery (3 hours recovery, right panels). Upper panels: open bars are total recycled ^{14}C-glucose d.p.m. in soleus glycogen; striped bars are recycled ^{14}C-glucose d.p.m. in soleus glycogen accounted for by the direct uptake of recycled ^{14}C-glucose from plasma. Lower panels: open bars are total soleus glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis (negative values do not appear on the graph). All data are expressed as means $\pm$ SEM. **significantly different from total recycled ^{14}C-glucose d.p.m. in soleus glycogen, *significantly different from rest, $^{+}$significantly different from no recovery ($P < 0.05$). $n = 6$ for all bars.

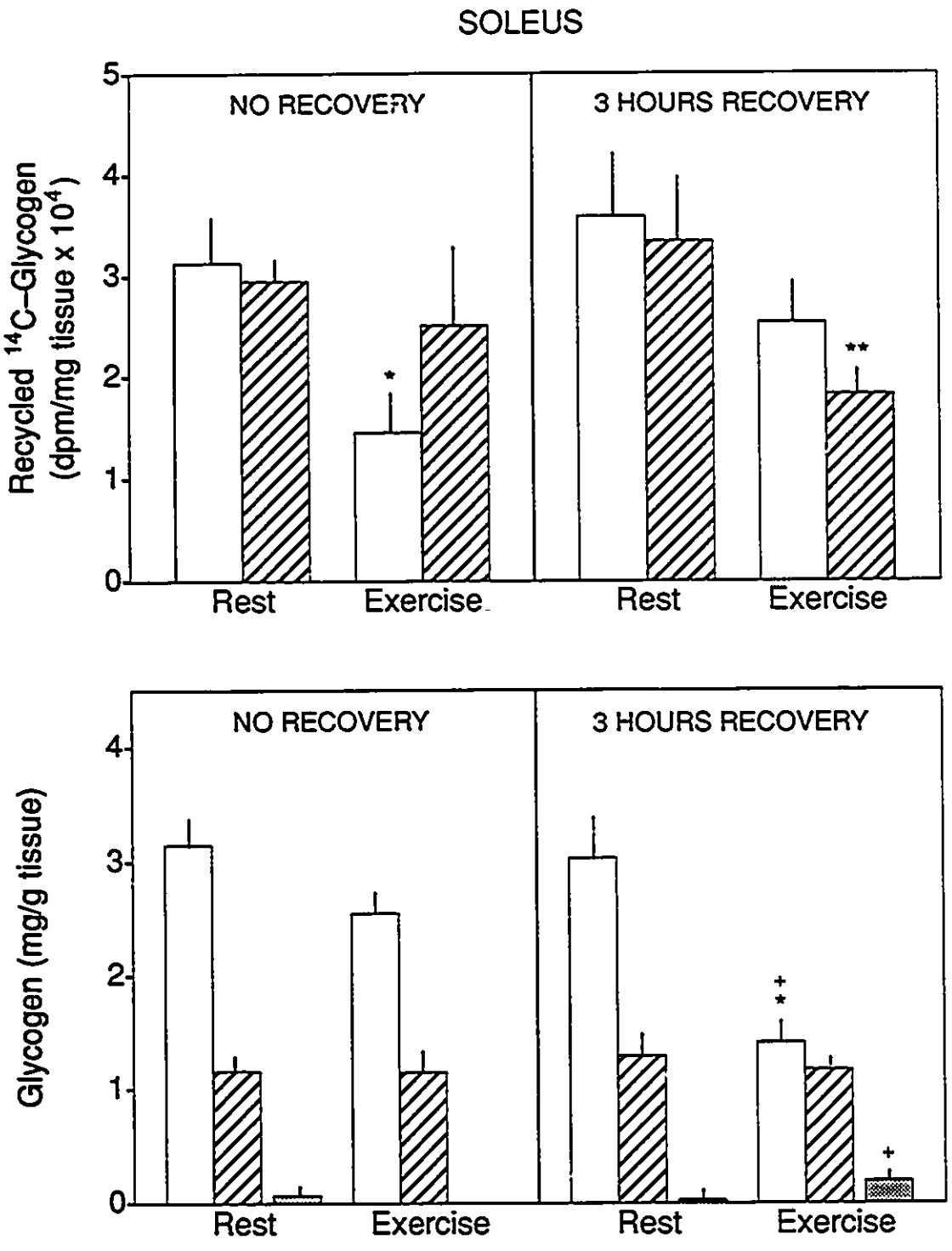

Figure 3.2: Soleus glycogen in rested and exercised rats infused with [^{3}H–3]– and [^{14}C–1]–glucose.

Soleus Glycogen (Figure 3.2)

Rest. Total glycogen in the soleus muscle was the same in the six-hour and nine-hour rested animals (3.15 ± 0.25 and 3.03 ± 0.38 mg/g, respectively, lower panels). There was a very high turnover of glycogen in the rested rats as indexed by both the high counts of recycled ^{14}C-glucose in glycogen (eg. $3.59 \times 10^4 \pm 0.65 \times 10^4$ d.p.m./mg muscle, upper right panel) as well as the amount of glycogen made via the direct uptake of plasma glucose (1.16 ± 0.13 mg/g and 1.29 ± 0.21 mg/g in six- and nine-hour rested animals, respectively, lower panels). This represents approximately 40% replacement of glycogen at rest. There were no significant differences between total recycled ^{14}C-glucose in glycogen and that which could be accounted for by the direct uptake of recycled ^{14}C-glucose from plasma (upper panels), suggesting that all of the glycogen was formed directly from plasma glucose. Dividing these nominal differences by plasma lactate specific activities indeed resulted in insignificant amounts of glycogen made by the glyconeogenic route in muscle (0.07 ± 0.10 and 0.03 ± 0.07 mg/g muscle in rested rats before and after the recovery period, respectively, lower panels).

Exercise (No Recovery). The prolonged swim had no apparent effect on the total amount of glycogen in the soleus (2.55 ± 0.19 mg/g, insignificant depletion, lower left panel) nor on the total amount of glycogen synthesized by the direct route (1.15 ± 0.25 mg/g) as compared to rested controls (lower left panel). Total recycled ^{14}C-glucose in glycogen, however, was significantly lower immediately following the

exercise period as compared to rested controls ($P < 0.05$). (This may be a methodological error as the direct incorporation of recycled ^{14}C-glucose into glycogen is greater than total recycled ^{14}C-glucose in glycogen, which is impossible.) Since the ^{14}C in glycogen arising from the direct uptake of recycled ^{14}C-glucose in plasma was greater than total recycled ^{14}C in glycogen, the corresponding calculated index of glyconeogenesis was a negative value and, thus, does not appear on the graph. Therefore, during exercise, it appears that all of the glycogen was synthesized from plasma glucose.

Postexercise Recovery. There was a net depletion of soleus glycogen during the three-hour postexercise recovery period as compared to both rested controls ($P < 0.05$) and exercised rats immediately following the swim ($P < 0.05$), despite the fact that direct glycogen synthesis was maintained (1.17 ± 0.13 mg/g) (lower left panel). While total recycled ^{14}C-glucose in glycogen was not different during postexercise recovery as compared to rest, it was significantly higher than ^{14}C-glycogen synthesized from recycled ^{14}C-glucose from plasma ($P < 0.05$, upper right panel). This difference translated into a small amount of gluconeogenically formed glycogen (0.20 ± 0.08 mg/g, lower right panel).

Figure 3.3: White gastrocnemius glycogen in rested and exercised rats infused with [^{3}H-3]- and [^{14}C-1]-glucose.
White gastrocnemius glycogen in rats infused with [^{3}H-3]- and [^{14}C-1]-glucose was assessed immediately following 4 hours of exercise or rest (no recovery, left panels) and after 3 hours of recovery (3 hours recovery, right panels). Upper panels: open bars are total recycled ^{14}C-glucose d.p.m. in white gastrocnemius glycogen; striped bars are recycled ^{14}C-glucose d.p.m. in white gastrocnemius glycogen accounted for by the direct uptake of recycled ^{14}C-glucose from plasma. Lower panels: open bars are total white gastrocnemius glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis. All data are expressed as means $\pm$ SEM. **significantly different from total recycled ^{14}C-glucose d.p.m. in white gastrocnemius glycogen, *significantly different from rest, $^{+}$significantly different from no recovery ($P < 0.05$). $n = 6$ for all bars.

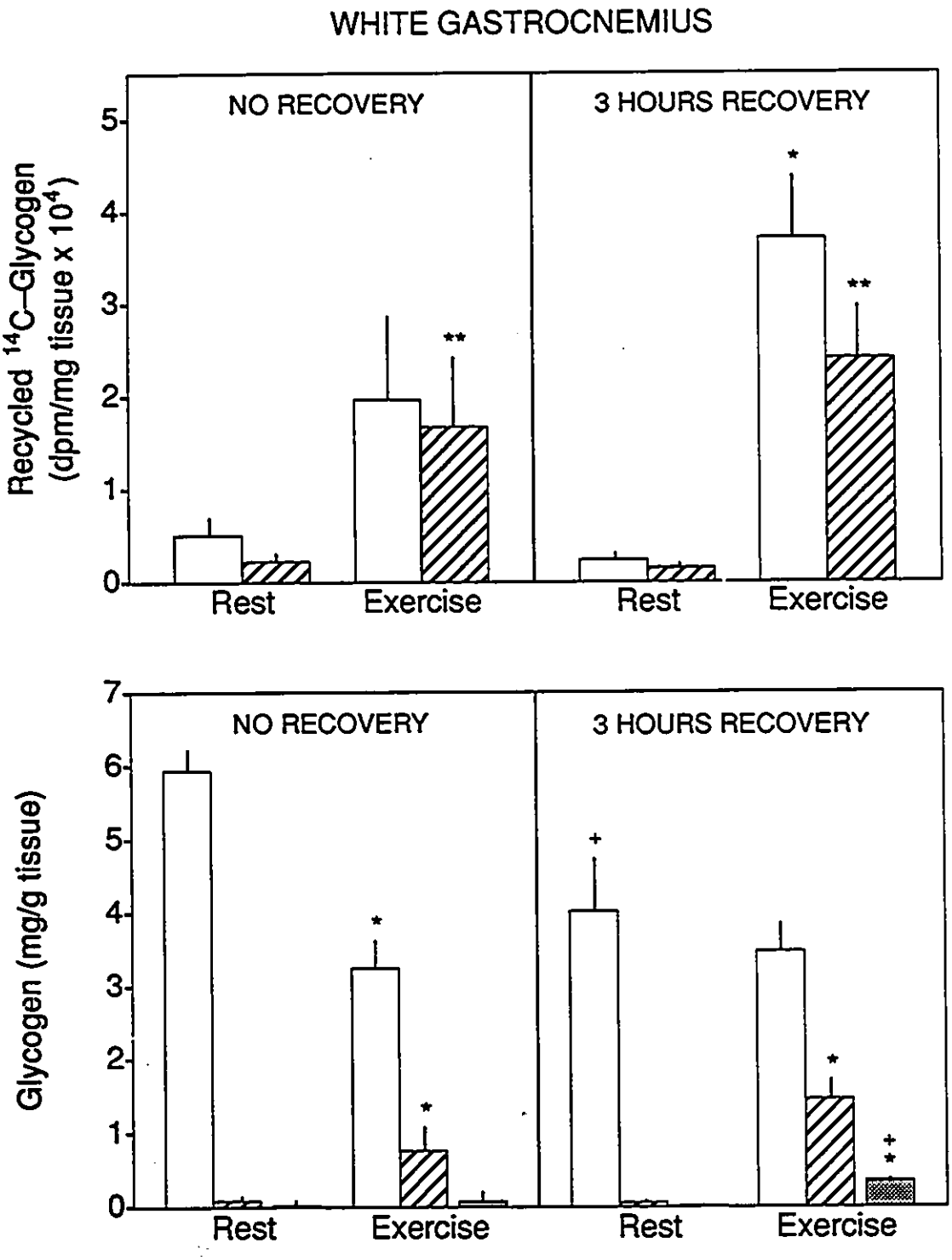

Figure 3.3: White gastrocnemius glycogen in rested and exercised rats infused with [^{3}H–3]– and [^{14}C–1]–glucose.

White Gastrocnemius Glycogen (Figure 3.3)

Rest. Total glycogen concentration in the white gastrocnemius muscle at rest (5.94 ± 0.30 mg/g, lower left panel) was double that of the soleus muscle. Following three more hours of rest, total glycogen was significantly lower than following six hours of rest ($P < 0.05$, lower right panel). Unlike the soleus muscle, glycogen turnover in the white gastrocnemius was very low at rest as reflected in the low incorporation of recycled ^{14}C-glucose into glycogen (eg. $0.24 \times 10^4 \pm 0.06 \times 10^4$ d.p.m./mg muscle in nine-hour rested rats, upper right panel) and in the extremely small amounts of glycogen synthesized from glucose (0.09 ± 0.03 and 0.06 ± 0.02 mg/g in six- and nine-hour rested animals, respectively, lower panels). As there were no statistically significant differences between total recycled ^{14}C-glucose in glycogen and that arising from plasma recycled ^{14}C-glucose, it follows that the corresponding indices of glyconeogenesis were barely detectable (0.03 ± 0.03 and 0.02 ± 0.01 mg/g muscle, lower panels).

Exercise (No Recovery). Exercise significantly depleted total glycogen in the white gastrocnemius (from 5.94 ± 0.30 to 3.25 ± 0.37 mg/g, $P < 0.05$, lower left panel) and, at the same, time stimulated glycogen synthesis. Total recycled ^{14}C-glucose in glycogen at the end of the prolonged swim was not significantly higher as compared to rested controls (upper left panel). Direct glycogen synthesis, however, did increase significantly during exercise (0.77 ± 0.31 mg/g, $P < 0.05$, lower left panel). Even though ^{14}C-glycogen synthesized directly from plasma recycled ^{14}C-glucose was

significantly lower than total recycled ^{14}C-glucose in glycogen ($P < 0.05$, upper left panel), dividing by the specific activity of plasma lactate resulted in an index of glyconeogenesis of only 0.08 ± 0.13 mg/g (lower left panel).

Postexercise Recovery. Total glycogen concentration following three hours of fasted postexercise recovery (3.47 ± 0.38 mg/g, lower right panel) was not different from that of the rested controls nor that immediately following the exercise period. Overall glycogen synthesis was stimulated during postexercise recovery as evidenced by the dramatic increase in total recycled ^{14}C-glucose incorporation into glycogen ($3.71 \times 10^4 \pm 0.68 \times 10^4$ d.p.m./mg muscle) over that of rested controls ($0.24 \times 10^4 \pm 0.06 \times 10^4$ d.p.m./mg, $P < 0.05$, upper right panel) as well as by the significant increase in direct glycogen synthesis (1.46 ± 0.27 mg/g) compared with nonexercised rats (0.06 ± 0.02 mg/g, $P < 0.05$, lower right panel). The significant difference between total and direct recycled ^{14}C-glucose in glycogen ($P < 0.05$, upper right panel) translated into a minimal uncorrected index of glyconeogenesis of 0.35 ± 0.04 mg/g (lower right panel), a value statistically significantly higher as compared to those at rest ($P < 0.05$) and immediately following exercise ($P < 0.05$) and representative of approximately 20% of total glycogen synthesis.

Figure 3.4: Red gastrocnemius glycogen in rested and exercised rats infused with [^{3}H-3]- and [^{14}C-1]-glucose.
Red gastrocnemius glycogen in rats infused with [^{3}H-3]- and [^{14}C-1]-glucose was assessed immediately following 4 hours of exercise or rest (no recovery, left panels) and after 3 hours of recovery (3 hours recovery, right panels). Upper panels: open bars are total recycled ^{14}C-glucose d.p.m. in red gastrocnemius glycogen; striped bars are recycled ^{14}C-glucose d.p.m. in red gastrocnemius glycogen accounted for by the direct uptake of recycled ^{14}C-glucose from plasma. Lower panels: open bars are total red gastrocnemius glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis. All data are expressed as means $\pm$ SEM. **significantly different from total recycled ^{14}C-glucose d.p.m. in red gastrocnemius glycogen, *significantly different from rest, $^{+}$significantly different from no recovery ($P < 0.05$). $n = 6$ for all bars.

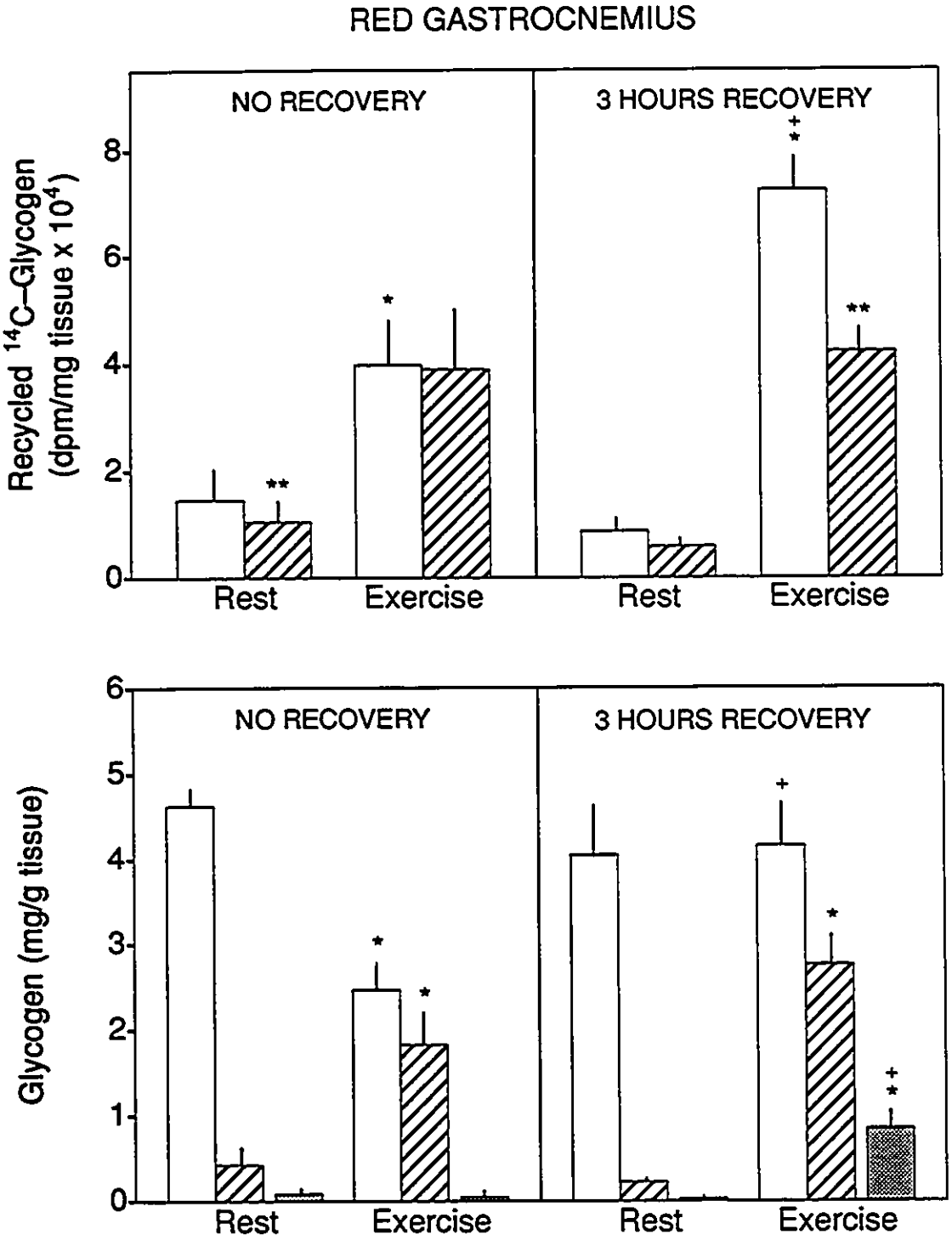

Figure 3.4: Red gastrocnemius glycogen in rested and exercised rats infused with [^{3}H–3]– and [^{14}C–1]–glucose.

Red Gastrocnemius Glycogen (Figure 3.3)

Rest. Total glycogen concentrations as well as direct glycogen synthesis in the red gastrocnemius muscle in resting animals were intermediate between those of the soleus and those of the white gastrocnemius. At rest, total glycogen concentration in the red gastrocnemius was 4.63 ± 0.20 mg/g muscle, a value between soleus and white gastrocnemius glycogen (lower left panel). As in the white muscle, glycogen turnover in the red gastrocnemius was low at rest; recycled ^{14}C-glucose incorporation into glycogen (upper left panel) was three times that of the white muscle, but still only a third that of the soleus. Direct glycogen synthesis was higher in the red gastrocnemius (0.43 ± 0.19 mg/g muscle, lower left panel) than in the white muscle but again, only about one third that in the soleus. Although there was a statistically significant difference between total and direct recycled ^{14}C-glycogen in red gastrocnemius in six-hour rested animals ($P < 0.05$, upper left panel), dividing by the specific activity of plasma lactate gave an uncorrected index of glyconeogenesis of only 0.09 ± 0.05 mg/g.

Exercise (No Recovery). Exercise both significantly decreased total glycogen in the muscle (from 4.63 ± 0.20 to 2.47 ± 0.32 mg/g, lower left panel) and significantly increased direct glycogen synthesis as indexed by total incorporation of recycled ^{14}C-glucose into glycogen (upper left panel) and the amount of glycogen synthesized from glucose (1.83 ± 0.37 mg/g, lower left panel), all $P < 0.05$ as compared to rested controls. The observation that total and direct recycled ^{14}C-glycogen were virtually

equal during exercise (upper left panel) suggests that all of the glycogen was made via the direct route; indeed, the corresponding glyconeogenic index was only 0.05 ± 0.07 mg/g.

Postexercise Recovery. Muscle glycogen was almost completely repleted during the three hours of fasted postexercise recovery. The 4.16 ± 0.51 mg/g glycogen in the red gastrocnemius was significantly higher than that immediately following the prolonged swim ($P < 0.05$, lower right panel). Total recycled ^{14}C-glycogen synthesis in the red muscle (upper right panel) was significantly increased over both rested controls ($P < 0.05$) and exercised rats ($P < 0.05$). Glycogen synthesized from glucose (2.76 ± 0.35 mg/g, lower right panel) was also significantly higher in rats recovering from the swim than that of nonexercised controls ($P < 0.05$). The significant difference between total recycled ^{14}C-glucose in glycogen and that which could be accounted for by the direct uptake of recycled ^{14}C-glucose from plasma (upper right panel) translated into a glyconeogenic index (0.85 ± 0.20 mg/g) significantly higher than the indices at rest ($P < 0.05$) and immediately following exercise ($P < 0.05$). This uncorrected index is a minimal estimate of the amount of glycogen formed via glyconeogenesis and represents approximately 24% of total glycogen synthesized during postexercise recovery.

DISCUSSION

TRACER APPROACH

The primary focus of this study was the question as to whether muscle repletes some or most of its glycogen from intramuscular lactate during the recovery period following prolonged swimming in rats. In spite of a large number of demonstrations of the possibility of glyconeogenesis (eg. indirect estimations based on substrate fluxes (3, 16) and label incorporation in perfusion studies (4, 23, 30, 32, 33)), the direct evidence under physiological circumstances is scarce and contradictory (6, 20). The few *in vivo* (6, 20) or perfusion tracer (23, 30, 32) studies examining the question of muscle glyconeogenesis that have used only [^{14}C-U]-lactate to detect the indirect pathway in muscle have found, as we have (Chapter 2), that although the potential for glyconeogenesis may be present, plasma lactate, at least, is not a major contributor to muscle glycogen repletion when plasma lactate levels are near normal. However, since there is ample evidence supporting at least the existence of the glyconeogenic pathway in skeletal muscle (3, 4, 6, 8, 14, 16, 18, 20, 23, 30, 32, 33), especially in conditions of high muscle lactate concentrations (eg. intense exercise in humans, refs. 3, 16; exogenous infusion into rats, ref. 20; or high lactate concentrations in the perfusate, refs. 23, 32), we hypothesized that lactate formed *locally* in the muscle could be contributing to glycogen synthesis. Since this lactate may be metabolized immediately within the muscle fibers or "shuttled" to other fibers within the muscle bed without entering the general circulation (5), it may not mix with the systemic lactate pool and, thus, may not be adequately traced using ^{14}C-lactate infusion into

plasma. Thus, glyconeogenesis from this source may not have been adequately characterized in these previous studies.

In an attempt to label intramuscular lactate more effectively, we infused [^{14}C-1]-glucose into plasma so that it might enter the muscles, be converted to ^{14}C-lactate and converted into glycogen within the muscle, labelling it with ^{14}C. But of course, ^{14}C-lactate would also leave the muscle, enter the plasma, undergo hepatic gluconeogenesis to form recycled ^{14}C-glucose which could then enter the plasma and be taken up directly into muscle glycogen, labelling it with recycled ^{14}C-glucose as well. Thus, the infusion of a second glucose tracer, [^{3}H-3]-glucose was necessary to account for the recycled ^{14}C-glucose units in glycogen arising from plasma. ([^{3}H-3]-glucose was used for the most part in place of [^{3}H-6]-glucose as the glucose recycling assay actually measures label in the sixth position.) In an attempt to quantitate the amount of glycogen formed from lactate, the differences between the total amount of recycled ^{14}C-glucose in glycogen and that arising from the direct uptake of plasma glucose were divided by the average specific activities of plasma ^{14}C-lactate during the recovery (or exercise) period. This is based on the assumption that ^{14}C-lactate specific activity in the plasma is similar to that of the muscle, which may or may not be true, especially following exercise. Brooks and Gaesser (6) have reported that following [^{14}C-U]-glucose injection into rats, specific activity of blood lactate was only slightly higher in rats that had exercised to exhaustion as compared to rest, while that of muscle lactate in exercised rats was approximately 3-10% higher than at rest. In addition, if intracellularly formed lactate does not equilibrate or mix with plasma

lactate, then the specific activities may be quite different. It is therefore reemphasized that the indices of glyconeogenesis are estimates and may not accurately reflect actual amounts of glycogen formed via the glyconeogenic route.

LIVER

Although hepatic glyconeogenesis is not the focus of the study and was included primarily as a verification of the methods used to detect glyconeogenesis, the pathways of repletion in the tissue warrant brief mention. As expected from previous findings (20, 21, 22, 24, 26, 27, Chapter 2), the indirect route is a major pathway of glycogen synthesis in liver despite the very low rates of glycogenesis in fasted rested and exercised rats and the preferential postexercise glycogen repletion in muscles (12, 13, 20, Chapter 2). While Johnson and Bagby (20) report 36% and 39% glycogen synthesis via the indirect route in livers of rested and exercised rats, respectively, with correction for label dilutions (17) in our study, glyconeogenesis from recycled glucose in the liver would constitute the predominant pathway for glycogenesis, close to doubling the contributions made by the direct route.

MUSCLE GLYCOGEN SYNTHESIS

The results of this chapter in terms of total and direct muscle glycogen synthesis and the effects of exercise and postexercise recovery on muscle glycogen depletion and repletion verify those of Chapter 2. The only inconsistency problem that should be addressed is the seemingly variable total glycogen concentration in the

white gastrocnemius in nine-hour rested animals. In Figure 2.1 (composite of animals from Chapters 2 and 3, p. 87), there is an apparent significant decrease in total white gastrocnemius glycogen concentration in rested animals during the three-hour recovery period. While this trend is also noticed in the individual series of rats infused with [^{14}C-U]-lactate (Series III, Chapter 2, Figure 2.4, p. 93) and [^{14}C-1]-glucose (Series II, Chapter 3, Figure 3.3, p. 124), rats infused with $NaH^{14}CO_3$ (Series II, Chapter 2, Figure 2.4) do not demonstrate this depletion of glycogen during rested recovery. This inconsistency is most likely due to the inherent variability of muscle glycogen concentrations dependent upon possible diurnal and seasonal effects, relative stress and activity levels of the animals during the fasted and experimental periods and the initial muscle glycogen concentrations in the individual animals. Otherwise, it can be seen once again that the soleus has a high rate of glycogen turnover at rest and is not depleted during prolonged swimming but rather in the postexercise recovery period; white and red gastrocnemii, on the other hand, show very low rates of glycogen synthesis at rest which are increased during exercise and more so during postexercise recovery to restore the exercise-induced depleted glycogen levels. With the results from the present study, it can be added that while the soleus appears to synthesize its glycogen from plasma glucose, glyconeogenesis from lactate formed locally in the muscle may contribute at least 20-25% to total glycogen formation in white and red gastrocnemii.

Increases in postexercise glyconeogenic activity in the muscles (gastrocnemii and possibly soleus) may be rooted in the exercise-induced changes in intramuscular

pH. Bonen et al. (4) have reported 49% and 39% increases in glyconeogenesis in perfused soleus and extensor digitorum longus muscles, respectively, when the pH of the perfusate was reduced from 7.4 to 6.5. While the mechanisms by which the increased acidity stimulates muscle glyconeogenesis have not yet been elucidated, they may be rooted in enhanced lactate transport into muscles (36) to provide additional glyconeogenic substrate and/or in enzymatic inhibition (phosphofructokinase) or stimulation (fructose-1,6-bisphosphatase) (4). Although it has been suggested that such decreases in intramuscular pH could be expected following prolonged exercise (31), it is unlikely in our prolonged swimming rat model as there is minimal lactate accumulation; however, since muscle pH was not determined in our swimming model, its potential stimulatory effects on glyconeogenesis cannot be ruled out as a possible contributing factor to postexercise glycogen synthesis.

WHITE AND RED GASTROCNEMII

The results indicate that the white and red gastrocnemii are capable of synthesizing a significant portion of their glycogen via muscle glyconeogenesis during both rest and recovery from prolonged submaximal exercise which is characterized by minimal plasma lactate accumulation. The source of this glyconeogenic substrate appears to be lactate (or pyruvate) formed locally in muscle, rather than plasma lactate. An estimate of the glyconeogenic contributions to glycogen synthesis in the gastrocnemius muscles was approximately 20-25% based on the uncorrected indices of glyconeogenesis. If significant dilution of the ^{14}C label from recycled [^{14}C-1]-glucose

does occur in the Krebs cycle in muscle as in liver (i.e. due to metabolic exchange of ^{14}C for ^{12}C in the OAA pool), the correction factor may be as high as that in the liver (1.5 to 2, refs. 17, 26). (This is only speculation, however, as, to our knowledge, there are no reports on the extent of OAA dilution in muscles to date.) Bonen et al. (4), as well as others (23, 30), have estimated that muscle glyconeogenesis (as traced by [^{14}C-U]-lactate) is underestimated by approximately 35-50% due presumably to label dilution by the glycolytic intermediates. Considering these underestimations, then, glyconeogenesis may be contributing as much as 30-40% of the glycogen formed in both red and white gastrocnemius muscles. This maximal 40% estimate of the contribution of glyconeogenesis to total muscle glycogen synthesis complements the report by Johnson and Bagby (20) that only about 50% of glycogen formed in the white gastrocnemius could be attributed to the direct uptake of plasma glucose in both exercised and nonexercised rats.

SOLEUS

The soleus muscle, on the other hand, appears to synthesize its glycogen primarily from glucose. The gluconeogenic contribution to glycogen synthesis in the soleus muscle (based on the uncorrected glyconeogenic index) was only about 2.3% in rested animals. Taking into account the 35-50% underestimation (4), the corrected glyconeogenic contribution to glycogen synthesis would correspond precisely to the 3.5-5% reported in perfused mouse soleus (4) as traced by [^{14}C-U]-lactate. These results are also in accordance with others (20, 23) who found relatively little glycone-

ogenic activity in soleus, presumably owing to very low levels of fructose-1,6-bisphosphatase activity in the muscle (23).

During recovery from exercise, however, there was an apparent increase in glyconeogenesis in the soleus muscle. As glyconeogenic activity in soleus was unexpected in light of previous reports (20, 23), there may be several possible explanations for such an apparent increase. The first is the inherent limitations in the experimental design. The glyconeogenic index is the result of the difference between one mean measurment of total recycled ^{14}C-d.p.m. in the muscle and the calculation of seven different means which is then divided by plasma lactate specific activity (which may not be truly reflective of intramuscular lactate specific activity). Thus, the potential errors in each measurement and in subsequent calculations must be taken into account. In addition, the calculation of the direct uptake of recycled ^{14}C-glucose in the soleus seems to be less consistent (as noted in the large errors, especially during exercise) which may also apply to the apparent glyconeogenesis during the postexercise period. (This inconsistency is manifested in Chapter 4, Figure 4.6, in lactate-loaded rats which showed evidence of glyconeogenic activity in the soleus at rest but not following exercise.) This could arise if the soleus did not synthesize its glycogen in a linear manner so that the average ratio of plasma ^{3}H-glucose to recycled ^{14}C-glucose during recovery would not apply. Indeed, the amount of glycogen synthesized by the direct route after one hour of recovery (preliminary data of $n = 3$ rats, not reported in this thesis) was approximately only 0.3 mg/g, significantly lower than that immediately following exercise and after three hours of postexercise

recovery, suggesting a nonlinear rate of glycogen synthesis over the three-hour recovery period.

Another possible reason for the notable but unexpected glyconeogenic contribution to glycogen is the fiber composition of the soleus muscle itself. The soleus is comprised of approximately 15% fast-twitch oxidative glycolytic fibers (FOG) (1, 2) which also constitute the majority of the red gastrocnemius muscle (1, 2). FOG fibers have been shown to have the highest glyconeogenic capacity (23) which may be further stimulated with prior exercise (Chapter 2). It may be, then, that prior prolonged swimming stimulated FOG fibers in the soleus muscle to synthesize a small amount of glycogen via glyconeogenesis. McLane and Holloszy (23) have also submitted that the slow but definite glyconeogenic rates observed in their perfused soleus muscles may be attributed to these fast-twitch oxidative fibers with a higher glyconeogenic capacity.

THE PATHWAY OF MUSCLE GLYCONEOGENESIS

The pathway by which lactate is converted to glycogen in muscle remains obscure. It has been proposed that pyruvate kinase could support the direct conversion of pyruvate into phosphoenolpyruvate (PEP) at rates which would correspond to glyconeogenic rates reported in muscle (10, 18, 23, 30), despite the high positive standard free energy of the reaction. Others, however, support the necessity of an enzymatic bypass to pyruvate kinase, as seen in liver. Because skeletal muscle does not possess significant activities of pyruvate carboxylase to transform pyruvate into

OAA as in liver (25), but does demonstrate significant activities of malic enzyme (NADP-dependent) and PEPCK (8, 9, 23, 25, 34), pyruvate could, theoretically, be converted to malate to OAA and then to PEP on its way to glycogen.

Although the present study does not shed any new light on the conversion of pyruvate to PEP in muscle in the pathway of glyconeogenesis, it indicates that the primary source of the lactate for glyconeogenesis in fast-twitch muscles is that formed locally in the muscles, rather than that taken up from plasma. This lactate may be formed within the same muscle fibers or possibly "shuttled" from adjacent fibers (5). Prior exercise seems to stimulate glyconeogenic activity in the muscle even in exercises not characterized by high plasma lactate accumulations (i.e. prolonged swimming, ref. 11, Chapter 1). This postexercise increase in glyconeogenic activity may be stimulated in part by *1)* decreases in intracellular pH (which may inhibit phosphofructokinase and/or stimulate fructose-1,6-bisphosphatase (4)), *2)* low glycogen levels, a potent stimulus for glycogenesis (7), or *3)* increased lactate transport into and/or between muscle fibers (5, 36) to provide additional glyconeogenic substrate.

REFERENCES

1. Ariano, M. A., R. B. Armstrong, and V. R. Edgerton. Hindlimb muscle fiber populations of five mammals. J. Histochem. Cytochem. 21(1): 51-55, 1973.

2. Armstrong, R. B., and R. O. Phelps. Muscle fiber type composition of the rat hindlimb. Am. J. Anat. 171: 259-272, 1984.

3. Åstrand, P. -O., E. Hultman, A. Juhlin-Dannfelt, and G. Reynolds. Disposal of lactate during and after strenuous exercise in humans. J. Appl. Physiol. 61(1): 338-343, 1986.

4. Bonen, A., J. C. McDermott, and M. H. Tan. Glycogenesis and glyconeogenesis in skeletal muscle: effects of pH and hormones. Am. J. Physiol. 258 (Endocrinol. Metab. 21): E693-E700, 1990.

5. Brooks, G. A. The lactate shuttle during exercise and recovery. Med. Sci. Sports Exerc. 18(3): 360-368, 1986.

6. Brooks, G. A., and G. A. Gaesser. End points of lactate and glucose metabolism after exhausting exercise. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(6): 1057-1069, 1980.

7. Conlee, R. K., R. C. Hickson, W. W. Winder, J. M. Hagberg, and J. O. Holloszy. Regulation of glycogen resynthesis in muscles of rats following exercise. Am. J. Physiol. 35(3): R145-R150, 1978.

8. Connett, R. J. Glyconeogenesis from lactate in frog striated muscle. Am J. Physiol. 237(5): C231-C236, 1979.

9. Crabtree, B., S. J. Higgins, and E. A. Newsholme. The activities of pyruvate carboxylase, phosphoenolpyruvate carboxylase and fructose diphosphatase in muscles from vertebrates and invertebrates. Biochem. J. 130: 391-396, 1972.

10. Dyson, R. D., J. M. Cardenas, and R. J. Barsotti. The reversibility of skeletal muscle pyruvate kinase and an assessment of its capacity to support glyconeogenesis. J. Biol. Chem. 250(9): 3316-3321, 1975.

11. Favier, R. J., H. E. Koubi, M. H. Mayet, B. Semporé, B. Simi, and R. Flandrois. Effects of gluconeogenic precursor flux alterations on glycogen resynthesis after prolonged exercise. J. Appl. Physiol. 63(5): 1733-1738, 1987.

12. Fell, R. D., J. A. McLane, W. W. Winder, and J. O. Holloszy. Preferential resynthesis of muscle glycogen in fasting rats after exhausting exercise. Am. J. Physiol. 238 (Regulatory Integrative Comp. Physiol. 7): R238-R332, 1980.

13. Gaesser, G. A., and G. A. Brooks. Glycogen repletion following continuous and intermittent exercise to exhaustion. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(4): 722-728, 1980.

14. Gleeson, T. Glycogen synthesis from lactate in skeletal muscle of the lizard *Dipsosaurus dorsalis*. J. Comp. Physiol. B. 156: 277-283, 1985.

15. Goodman, M. N., R. Dietrich, and P. Luu. Formation of gluconeogenic precursors in rat skeletal muscle during fasted-refed transition. Am. J. Physiol. 259 (Endocrinol. Metab. 22): E513-E516, 1990.

16. Hermansen, L., and O. Vaage. Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. Am J. Physiol. 233(5): E422-E429, 1977.

17. Hetenyi, G., Jr. Correction for the metabolic exchange of ^{14}C for ^{12}C atoms in the pathway of gluconeogenesis *in vivo*. Fed. Proc. 41: 104-109, 1982.

18. Hiatt, H. H., M. Goldstein, J. Laureau, and B. L. Horecker. The pathway of hexose synthesis from pyruvate in muscle. J. Biol. Chem. 231: 303-307, 1958.

19. Hutber, C. A., and A. Bonen. Glycogenesis in muscle and liver during exercise. J. Appl. Physiol. 66(6): 2811-2817, 1989.

20. Johnson, J. L., and G. J. Bagby. Gluconeogenic pathway in liver and muscle glycogen synthesis after exercise. J. Appl. Physiol. 64(4): 1591-1599, 1988.

21. Katz, J., and J. D. McGarry. The glucose paradox: is glucose a substrate for liver metabolism? J. Clin. Invest. 74: 1901-1909, 1984.

22. Lang, C. H., G. J. Bagby, H. L. Blakesley, J. L. Johnson, and J. J. Spitzer. Plasma glucose concentration determines direct *versus* indirect liver glycogen synthesis. Am. J. Physiol. 251 (Endocrinol. Metab. 14): E584-E590, 1986.

23. McLane, J. A., and J. O. Holloszy. Glycogen synthesis from lactate in the three types of skeletal muscle. J. Biol. Chem. 254: 6548-6553, 1979.

24. Mitrakou, A., R. Jones, Y. Okuda, J. Pena, N. Nurjhan, J. B. Field, and J. E. Gerich. Pathway and carbon sources for hepatic glycogen repletion in dogs. Am. J. Physiol. 260 (Endocrinol. Metab. 23): E194-E202, 1991.

25. Opie, L. H., and E. A. Newsholme. The activities of fructose 1,6-diphosphatase, phosphofructokinase and phosphoenolpyruvate carboxykinase in white muscle and red muscle. Biochem. J. 103: 391-399, 1967.

26. Radziuk, J. Sources of carbon in hepatic glycogen synthesis during absorption of an oral glucose load in humans. Fed. Proc. 41:110-116, 1982.

27. Radziuk, J. Tracer methods and the metabolic disposal of a carbohydrate load in man. Diabetes/Metabolism Reviews 3(1): 231-267, 1987.

28. Reeves, R. E. The estimation of primary carbinol groups in carbohydrates. J. Amer. Chem. Soc. 63: 1476-1477, 1941.

29. Rodbard, D. Statistical estimation of the minimal detectable concentration ("sensitivity") for radioligand assays. Anal. Biochem. 90: 1-12, 1978.

30. Shiota, M., S. Golden, and J. Katz. Lactate metabolism in the perfused rat hindlimb. Biochem. J. 222: 281-292, 1984.

31. Spriet, L. L., K. Soderlund, M. Bergstom, and E. Hultman. Skeletal muscle glycogenolysis, glycolysis, and pH during electrical stimulation in men. J. Appl. Physiol. 62: 616-621, 1987.

32. Stevenson, R. W., D. R. Mitchell, G. K. Hendrick, R. Rainey, A. D. Cherrington, and R. T. Frizzell. Lactate as substrate for glycogen resynthesis after exercise. J. Appl. Physiol. 62(6): 2237-2240, 1987.

33. Talmadge, R. J., J. I. Scheide, and H. Silverman. Glycogen synthesis from lactate in chronically active muscle. J. Appl. Physiol. 66(5):2231-2238, 1989.

34. Talmadge, R. J., and H. Silverman. Glyconeogenic and glycogenic enzymes in chronically active and normal skeletal muscle. J. Appl. Physiol. 71(1): 182-191, 1991.

35. Terjung, R. L., K. M. Baldwin, W. W. Winder, and J. O. Holloszy. Glycogen repletion in different types of muscle and in liver after exhausting exercise. Am. J. Physiol. 226(6): 1387-1391, 1974.

36. Watt, P. W., P. A. MacLennan, H. S. Hundal, C. M. Kuret and M. J. Rennie. L(+)-Lactate transport in perfused rat skeletal muscle: kinetic characteristics and sensitivity to pH and transport inhibitors. Biochim. Biophys. Acta 944: 213-222, 1988.

CHAPTER 4: EFFECT OF POSTEXERCISE LACTATE INFUSION ON MUSCLE AND LIVER GLYCOGEN SYNTHESIS

INTRODUCTION

To date, the availability of high muscle concentrations of lactate has been considered necessary for significant muscle glycogen synthesis via the gluconeogenic route (glyconeogenesis). The few *in vivo* human studies (3, 18) supporting postexercise muscle glyconeogenesis used high intensity exercise models (running or cycling) which are characterized by high lactate production and accumulation (plasma levels up to 20 mM, ref. 18). Following simulated high intensity exercise (electrical stimulation and epinephrine), significant glycogen synthesis from lactate in the perfused rat muscle occurred only when the perfusate contained high lactate concentrations (10.5 or 18 mM) (38). Moreover, postexercise gluconeogenic glycogen synthesis has been reported in white muscle in rat but only in the presence of elevated lactate levels produced by nutritive support (22).

In contrast to previous studies, we have found that both white and red gastrocnemius muscles replete some of their glycogen via the glyconeogenic route (primarily from lactate formed locally in the muscles) during a fasted recovery period following prolonged swimming, an exercise model not associated with high lactate accumulation (1.3 mM lactate in plasma at the end of four hours of exercise). The purposes of the studies presented in this chapter are, therefore, *i)* to establish the consistency of our results with those previously published by demonstrating glyconeogenesis in the recovery period in the presence of high lactate, *ii)* to examine the interactions in

glycogen synthesis between exercise, *per se*, (submaximal and, therefore, with minimal lactate accumulation) and additional lactate which would simulate that formed after more intense exercise, and *iii)* to determine whether any effects of lactate demonstrated could be explained by the additional glucose synthesized by the liver in response to the increase in substrate concentrations.

METHODS

EXPERIMENTAL PROTOCOL

Three series of rats (rested controls and swimmers) were infused with either *i)* $NaH^{14}CO_3$, *ii)* [^{14}C-U]-lactate or *iii)* [^{14}C-1]-glucose (at rates previously described) and followed experimental protocols as described in Chapters 1, 2 and 3. The only difference was the infusion of a 15% one-third neutralized lactic acid solution (27) at a rate of 0.055 ml/min (8.25 mg/min) in place of saline during the three-hour recovery period in all series of rats. This lactic acid solution was infused rather than sodium lactate so as to avoid the potential increases in blood flow and extracellular fluid volume (see ref. 7) as well as progressive alkalosis characteristic of sodium lactate infusions, presumably due to the accumulations of sodium ions (7). $NaH^{14}CO_3$-lactic acid infused rats were included as controls (as no incorporation of ^{14}C is expected in muscle glycogen as previously discussed), as well as for the determination of the minimal detectable differences for statistical analyses in lactate-infused rats. Rats infused with [^{14}C-1]-glucose and saline during recovery (Chapter 3) were plotted on the graphs as "controls" for load effects and for statistical compari-

sons.

In order to assess whether the effects of lactic acid infusion on glycogen formation were due to lactate itself or rather to the resulting plasma glucose from hepatic gluconeogenesis, a fourth series of rats (both rested and exercised) was infused with [^{14}C-1]-glucose (as above) and a 3% glucose load at 0.055 ml/min (1.7 mg/min) during recovery. The concentration of the glucose load was determined by infusing glucose solutions of various concentrations into resting rats and assessing which produced comparable plasma glucose concentrations to those attained in lactic acid-infused rested rats.

CALCULATIONS

Total and radiolabelled plasma glucose, plasma lactate and tissue glycogen were quantitated, and direct glycogen synthesis, direct ^{14}C-glycogen and the indices of glyconeogenesis were calculated as described in Chapters 1, 2 and 3 for NaH$^{14}CO_3$-, [^{14}C-U]-lactate- and [^{14}C-1]-glucose-infused rats, respectively. Because the specific activities of plasma lactate in rats infused with [^{14}C-1]-glucose and lactate would not reflect intramuscular lactate specific activities (due to the high concentrations of cold lactate in plasma which are not expected to equilibrate completely with the intramuscular lactate pool), the indices of glyconeogenesis in these rats were calculated by dividing the differences between total and direct recycled ^{14}C-glucose in glycogen by the average specific activity of plasma ^{14}C-glucose as the latter is the precursor to ^{14}C-lactate in muscle. This calculation, then, would represent a minimal, uncorrected

estimate of the index of gluconeogenic glycogen formation.

STATISTICAL ANALYSIS

Statistical analyses on plasma data were done using Dunn's procedure for *a priori* planned multiple comparisons. Significant differences between total and direct (recycled) ^{14}C-glycogen in liver and muscle were detected using the minimal detectable difference (MDD) test as described in Chapter 2. The MDD used for the test in lactic acid-infused rats was that calculated from equation 2.4 (p. 83), where m_o was the average of the differences between total and direct ^{14}C-d.p.m. in glycogen in the three muscles in rats infused with $NaH^{14}CO_3$ and cold lactic acid. In both rested and exercised rats infused with [^{14}C-1]-glucose and either saline, glucose or lactic acid during recovery, total recycled ^{14}C-glucose in glycogen, total tissue glycogen, glycogen synthesized by the direct uptake of plasma glucose and the indices of glyconeogenesis were compared with respect to the effects of both exercise and loads using analysis of variance with a completely randomized factorial design and an *a posteriori* comparison by Scheffe's procedure. The significant effects of exercise on total ^{14}C-glycogen, total glycogen concentrations, the amounts of glycogen synthesized via the direct route and the indices of glyconeogenesis in both [^{14}C-U]-lactate and $NaH^{14}CO_3$-infused rats, respectively, were detected using Student's *t* tests. The level of significance was set at $P < 0.05$ for all tests.

RESULTS

Plasma Lactate (Figure 4.1)

Plasma lactate concentrations in swimming rats were not significantly different from those of rested controls before the exercise period in animals infused with glucose or lactic acid during recovery. During exercise, lactate concentrations were higher as previously seen (Chapter 1) although this was not significant in one series (Figure 4.1, lower panel) because of another problem (see below). Sixty minutes of lactic acid infusion dramatically increased plasma lactate concentrations from 0.56 $\pm$ 0.03 mM and 1.34 $\pm$ 0.24 mM immediately following the exercise period to 12.39 $\pm$ 0.75 mM and 12.87 $\pm$ 1.66 mM in rested and exercised rats, respectively (upper curve). Lactic acid infusion caused a sharper increase in plasma lactate concentrations in exercised rats as compared to rested controls, so that following three hours of loading, differences between rested (14.71 $\pm$ 1.20 mM) and exercised animals (21.50 $\pm$ 4.11 mM) reached significance.

In animals which received a postexercise glucose load (lower graph), there was an apparent large increase in plasma lactate (to 3.22 $\pm$ 1.73 mM) following one hour of swimming. This apparent increase was due to one single sample of 11.78 mM, which may have been the result of either maximal exertion or severe stress during the first hour of swimming in the rat, or a contamination of the sample. Disregarding the sample gives a mean plasma lactate level of 1.55 $\pm$ 0.30 mM, which is in keeping with previous results (upper graph and Chapter 1 results). Glucose loading during recovery did not result in significant increases in plasma lactate concentrations in

either rested controls or exercised animals; moreover, plasma lactate concentrations were identical in glucose-loaded rested (1.06 $\pm$ 0.14 mM) and exercised animals (0.96 $\pm$ 0.62 mM) following one hour of loading and throughout the recovery period despite the initially higher lactate (not significant) in exercised (1.00 $\pm$ 0.06 mM) as compared to rested controls (0.56 $\pm$ 0.03 mM) immediately following the swim.

Figure 4.1: Plasma lactate concentrations in rested and exercised rats infused with cold lactic acid or glucose during the recovery period.
Plasma lactate concentrations were determined in rats during a two-hour basal period (0-120 minutes), four hours of swimming or rest (120-360 minutes) and three hours (360-540 minutes) of a postexercise lactic acid (upper graph) or glucose infusion (lower graph). Solid lines represent the exercised group and the dashed lines, the rested controls. All data are expressed as means $\pm$ SEM. *significantly different from rest ($P < 0.05$). $n = 6$ for each point on the curves.

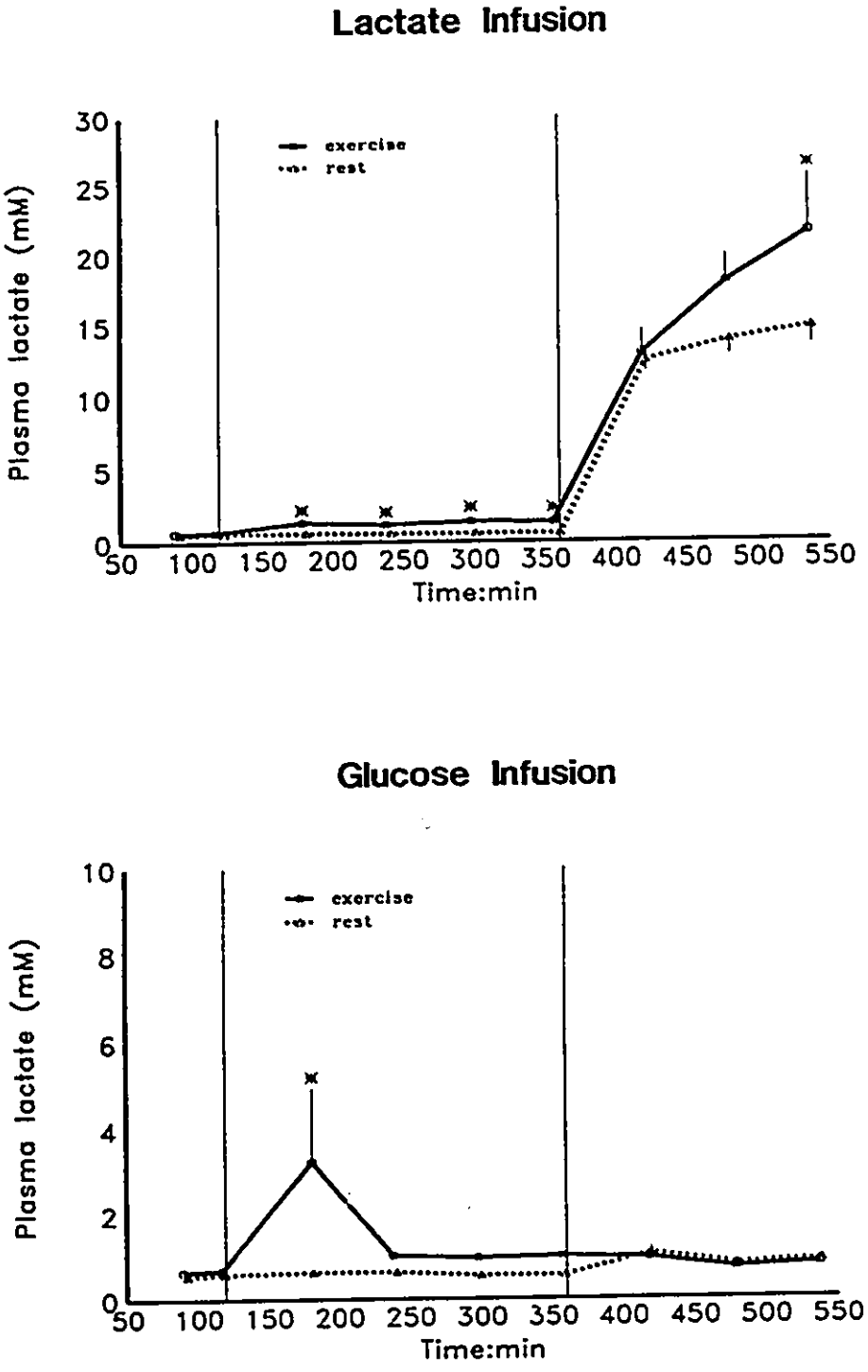

Figure 4.1: Plasma lactate concentrations in rested and exercised rats infused with cold lactic acid or glucose during the recovery period.

Figure 4.2: Plasma glucose concentrations in rested and exercised rats infused with cold lactic acid or glucose during the recovery period.
Plasma glucose concentrations were determined in rats during a two-hour basal period (0-120 minutes), four hours of swimming or rest (120-360 minutes) and three hours (360-540 minutes) of a postexercise lactic acid (upper graph) or glucose infusion (lower graph). Solid lines represent the exercised group and the dashed lines, the rested controls. All data are expressed as means $\pm$ SEM. *significantly different from rested control ($P < 0.05$). $n = 6$ for each point on the curves.

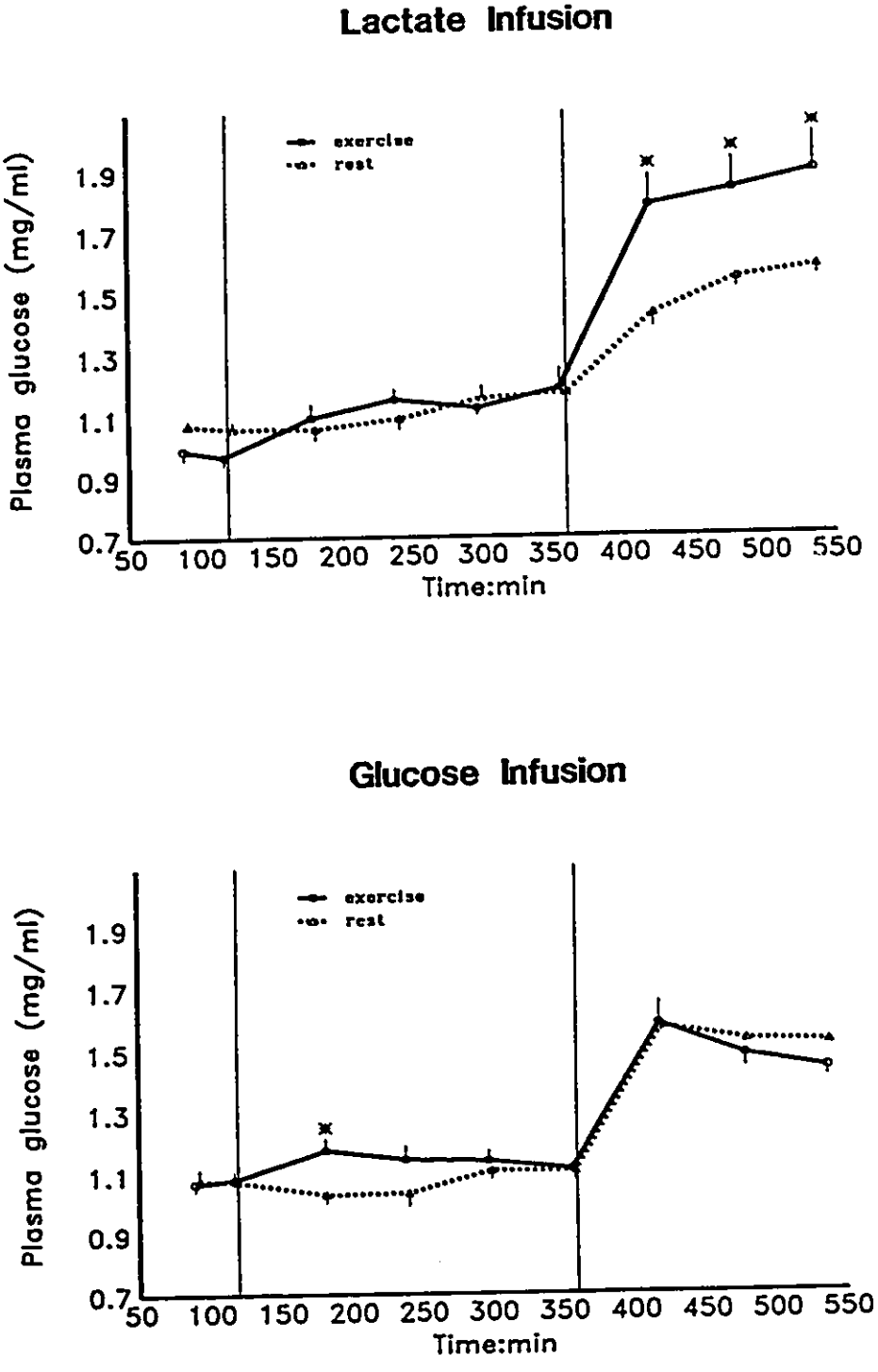

Figure 4.2: Plasma glucose concentrations in rested and exercised rats infused with cold lactic acid or glucose during the recovery period.

Plasma Glucose (Figure 4.2)

Although swimming caused a slight increase in plasma glucose concentrations (eg. from 0.97 ± 0.03 mg/ml to 1.10 ± 0.05 mg/ml after one hour of swimming, upper graph), glucose levels throughout the four-hour exercise were not different from those of rested controls (upper and lower graphs). (The apparent significant difference in the lower graph was again due to a very high plasma glucose level in only one rat sample after one hour of swimming.) Plasma glucose in exercised rats increased from 1.19 ± 0.06 mg/ml immediately postexercise to 1.78 ± 0.10 mg/ml following one hour of a lactate-loaded recovery, a level significantly higher than that of lactic acid-infused rested controls, and remained significantly higher than controls for the three-hour recovery period.

Glucose was infused into rats during recovery in attempt to match plasma glucose levels attained in lactic acid-infused rested controls, so as to ascertain whether lactate was exerting effects on its own or via resulting increases in plasma glucose. Indeed, plasma glucose concentrations attained in glucose-infused rested controls (eg. 1.53 ± 0.01 mg/ml, two hours of recovery) were very similar to those of lactate-loaded rats (eg. 1.54 ± 0.03 mg/g, two hours of recovery) throughout recovery. Plasma glucose levels in glucose-loaded exercised and nonexercised rats were both 1.58 mg/ml after one hour postexercise, and did not change significantly in either group throughout recovery.

Figure 4.3: ^{14}C-Glycogen in liver after either saline, glucose or lactic acid infusion during recovery.
^{14}C-glucose in liver glycogen was determined in rats following recovery from 4 hours of exercise (exercise + recovery, lower graph) or rest (rest + recovery, upper graph). Rats infused with [^{3}H-3]- and [^{14}C-1]-glucose were also infused with either saline, glucose or lactic acid during the three-hour recovery period. $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats were infused with lactic acid during the recovery period. Open bars are total ^{14}C-glucose d.p.m. in liver glycogen (total recycled ^{14}C-glucose d.p.m. in the cases of [^{14}C-1]-glucose infusion); striped bars are ^{14}C-glucose d.p.m. in liver glycogen accounted for by the direct uptake of plasma ^{14}C-glucose (recycled ^{14}C-glucose in [^{14}C-1]-glucose-infused rats). All data are expressed as means $\pm$ SEM. **significantly different from total (recycled) ^{14}C-glucose d.p.m. in liver glycogen; $^{+}$significantly different from saline infusion and $^{++}$significantly different from glucose infusion (for [^{14}C-1]-glucose-infused rats only), ($P < 0.05$). $n = 6$ for all bars except $NaH^{14}CO_3$-infused exercised rats where $n = 8$.

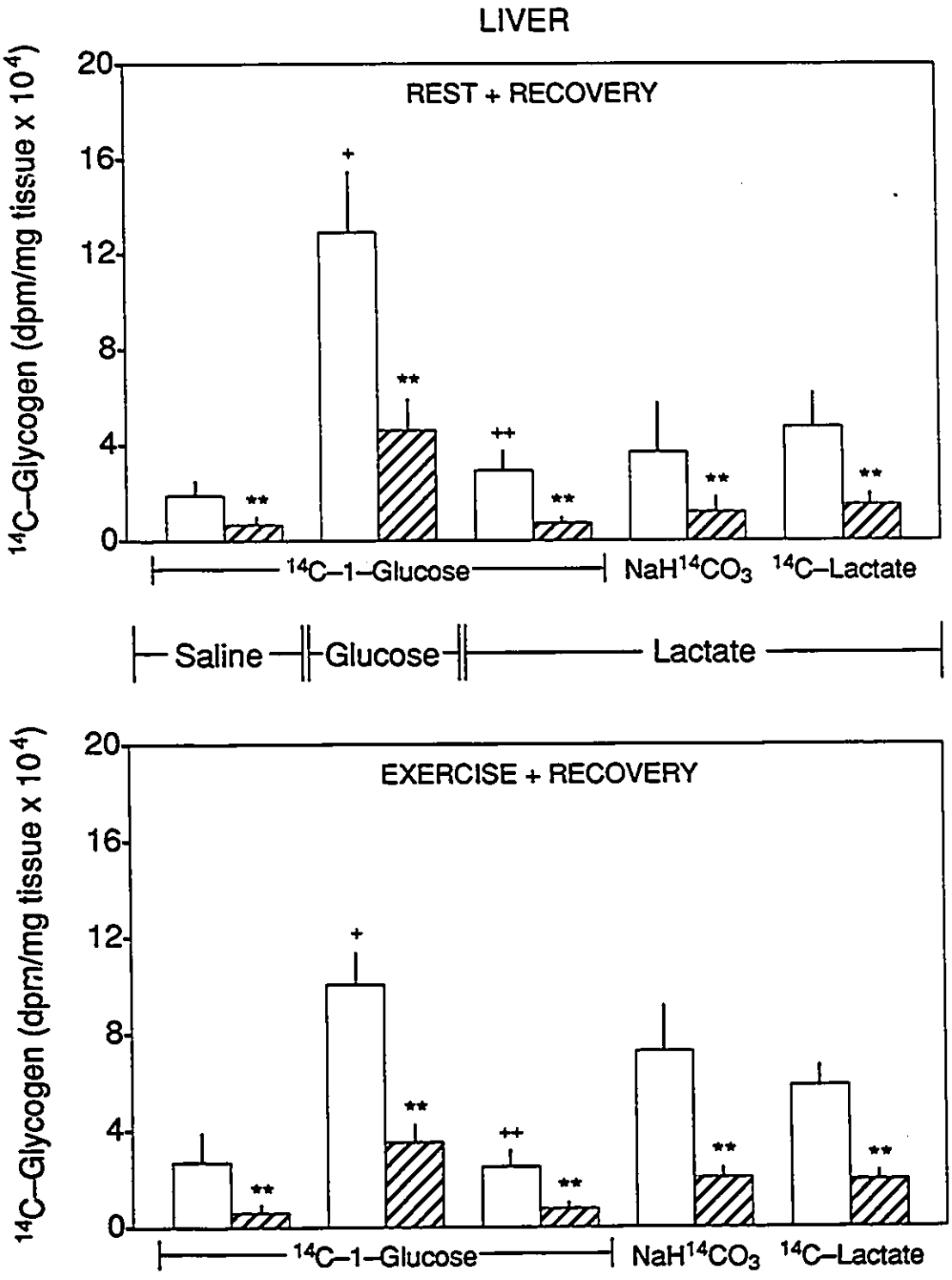

Figure 4.3: ^{14}C–Glycogen in liver after either saline, glucose or lactic acid infusion during recovery.

Figure 4.4: Glycogen in liver after either saline, glucose or lactic acid infusion during recovery.
Liver glycogen was assessed in rats following recovery from 4 hours of exercise (exercise + recovery, lower graph) or rest (rest + recovery, upper graph). Rats infused with [^{3}H-3]- and [^{14}C-1]-glucose were also infused with either saline, glucose or lactic acid during the three-hour recovery period. $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats were infused with lactic acid during the recovery period. Open bars are total liver glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis. All data are expressed as means $\pm$ SEM. For the same glycogen parameters: *significantly different from rest, $^{+}$significantly different from saline infusion and $^{++}$significantly different from glucose infusion (for [^{14}C-1]-glucose infusions only), ($P < 0.05$). $n = 6$ for all bars except $NaH^{14}CO_3$-infused exercised rats where $n = 8$.

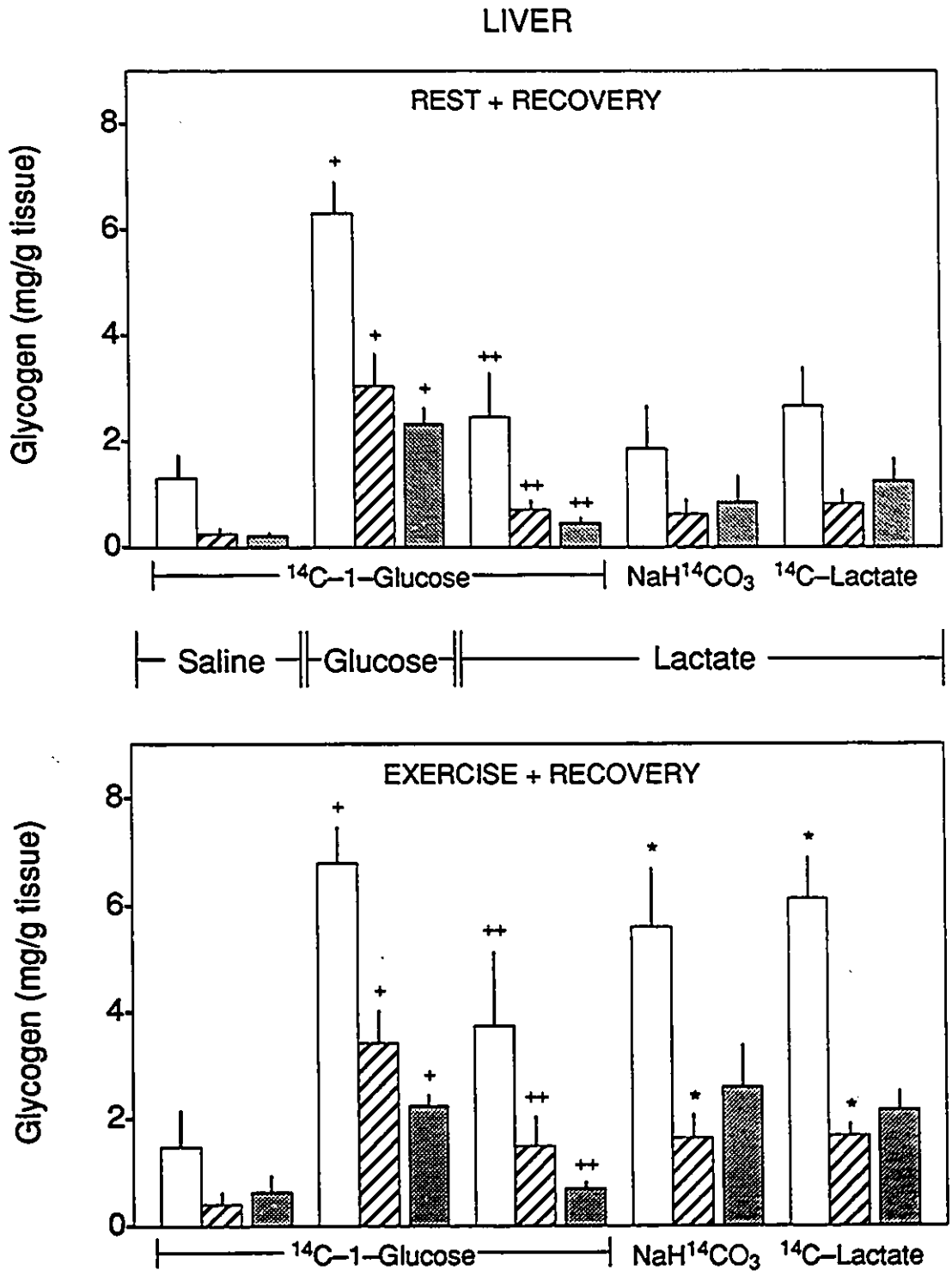

Figure 4.4: Glycogen in liver after either saline, glucose or lactic acid infusion during recovery.

^{14}C-Glycogen in Liver (Figure 4.3)

Rest (Upper Graph). Hepatic glycogen synthesis in rested fasted rats was low as indexed by the small amount of total recycled ^{14}C-glucose incorporated into glycogen in saline-infused animals ($1.90 \times 10^4 \pm 0.57 \times 10^4$ d.p.m./g liver). Glucose infusion into rested rats, however, significantly increased recycled ^{14}C-glucose incorporation into liver glycogen as compared to those resulting from both saline and lactic acid infusions ($P < 0.05$). In fact, exogenous lactic acid had no significant effect on total recycled ^{14}C-glucose incorporation into glycogen as compared to saline-infused rats. With each gluconeogenic tracer with or without exogenous substrate infusion, less than half of the total (recycled) ^{14}C-glucose in glycogen could be accounted for by the direct uptake of (recycled) plasma ^{14}C-glucose, indicative of significant gluconeogenic contributions to glycogenesis in the liver, consistent with previous findings (14, 22, 23, 24, 26, 28, 29, 32, 33, 36, 42, Chapters 2 and 3).

Postexercise Recovery (Lower Graph). Prolonged swimming had no statistically significant effects on total (recycled) ^{14}C-glucose incorporation into liver glycogen during postexercise recovery in saline-, glucose- or lactic acid-infused rats as compared to respective rested controls. In fact, the relative pattern in exercised rats was very similar to that of the rested controls. As in rested animals, glucose loading in exercised rats significantly increased total recycled ^{14}C-glucose incorporation into liver glycogen over those of saline- and lactic acid-infused rats ($P < 0.05$), whereas lactate-loading had no apparent significant effect on label incorporation. Again, less

than half of the total (recycled) ^{14}C-glycogen could be attributed to (recycled) plasma glucose in [^{14}C-1]-glucose, $NaH^{14}CO_3$ and [^{14}C-U]-lactate trials, suggesting that a major fraction of the glycogen synthesized was from gluconeogenic precursors.

Liver Glycogen Concentrations (Figure 4.4)

Rest (Upper Graph). Total hepatic glycogen and that synthesized by both direct and indirect routes in rested rats were significantly elevated with glucose infusion. Total glycogen concentration following three hours of glucose infusion into rested rats (6.30 $\pm$ 0.59 mg/g) was significantly higher than total glycogen following saline (1.31 $\pm$ 0.40 mg/g, $P < 0.05$) and lactic acid infusions (2.45 $\pm$ 0.82 mg/g, $P < 0.05$). The amount of glycogen synthesized by the direct uptake of glucose was also significantly increased with glucose infusion (3.04 $\pm$ 0.58 mg/g) as compared to saline (0.26 $\pm$ 0.09 mg/g) and lactic acid infusions (0.71 $\pm$ 0.15 mg/g) as were the uncorrected indices of glyconeogenesis (2.32 $\pm$ 0.31, 0.22 $\pm$ 0.06 and 0.46 $\pm$ 0.10 mg/g liver in glucose-, saline- and lactic acid-infused rats, respectively), (all $P < 0.05$, glucose *versus* saline or lactate). The indices of glyconeogenesis in lactate-loaded rats as traced using $NaH^{14}CO_3$ (0.85 $\pm$ 0.47 mg/g) and [^{14}C-U]-lactate infusions (1.26 $\pm$ 0.43 mg/g) appear higher than that using [^{14}C-1]-glucose. It should be noted that the glyconeogenic index in lactate-loaded rats in the [^{14}C-1]-glucose trial was calculated using average plasma ^{14}C-glucose specific activity and would represent a minimal estimate. Calculation of the index based on the plasma specific activities of ^{14}C-lactate gives a glyconeogenic index of 2.07 $\pm$ 0.60 mg/g. Using either $NaH^{14}CO_3$ or

[^{14}C-U]-lactate, the uncorrected indices of glyconeogenesis amounted to more glycogen made via the indirect route than that formed directly from glucose.

Postexercise Recovery (Lower Graph). While prior prolonged swimming tended to increase total, direct and gluconeogenic glycogen to some extent, major increases (in total and direct glycogen) were noted only in lactate-loaded exercised rats. These differences were significant in the studies where either $NaH^{14}CO_3$ or [^{14}C-U]-lactate were used as tracers. Total glycogen rose to 5.59 $\pm$ 1.10 and 6.13 $\pm$ 0.74 mg/g, while the amount of glycogen formed via the direct route was 1.65 $\pm$ 0.43 and 1.69 $\pm$ 0.23 mg/g in $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats, respectively, $P < 0.05$ as compared to respective rested controls. The uncorrected indices of glyconeogenesis were not statistically significantly higher than those of rested controls following three hours of postexercise lactic acid infusion (2.60 $\pm$ 0.77 and 2.18 $\pm$ 0.34 mg/g in $NaH^{14}CO_3$ and [^{14}C-U]-lactate trials, respectively) but did amount to more glycogen than that made via the direct route. Total (3.74 $\pm$ 1.38 mg/g), direct (1.27 $\pm$ 0.40 mg/g) and gluconeogenic glycogen (0.63 $\pm$ 0.13 mg/g) in [^{14}C-1]-glucose-infused rats which were lactate-loaded during postexercise recovery, however, were not different from rested controls and were each lower than those of the other two lactate trials. Dividing by the average plasma specific activity of ^{14}C-lactate in lactate-loaded exercised rats infused with [^{14}C-1]-glucose gives a glyconeogenic index of 2.48 $\pm$ 0.34 mg/g, which is more comparable to those in the $NaH^{14}CO_3$ and [^{14}C-U]-lactate trials and, in the liver, likely more appropriate. Individual variations in

hepatic glycogen are most likely the cause of these inconsistencies in total and direct glycogen values (particularly in the study with lactate loading after exercise and [^{14}C-1]-glucose infusion). With glucose infusion, prior exercise had no apparent effect on the amounts of glycogen formed in the liver as compared to glucose-infused rested controls. Total (6.79 $\pm$ 0.66 mg/g), direct (3.42 $\pm$ 0.58 mg/g) and gluconeogenic glycogen (2.24 $\pm$ 0.18 mg/g) in these glucose-loaded rats were significantly higher ($P < 0.05$) than those which received saline (1.49 $\pm$ 0.66, 0.42 $\pm$ 0.18 and 0.65 $\pm$ 0.31 mg/g for total, direct and gluconeogenic glycogen, respectively) or lactic acid during recovery.

Figure 4.5: ^{14}C-Glycogen in soleus after either saline, glucose or lactic acid infusion during recovery.
^{14}C-glucose in soleus glycogen was determined in rats following recovery from 4 hours of exercise (exercise + recovery, lower graph) or rest (rest + recovery, upper graph). Rats infused with [^{3}H-3]- and [^{14}C-1]-glucose were also infused with either saline, glucose or lactic acid during the three-hour recovery period. $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats were infused with lactic acid during the recovery period. Open bars are total ^{14}C-glucose d.p.m. in soleus glycogen (total recycled ^{14}C-glucose d.p.m. in the cases of [^{14}C-1]-glucose infusion); striped bars are ^{14}C-glucose d.p.m. in soleus glycogen accounted for by the direct uptake of plasma ^{14}C-glucose (recycled ^{14}C-glucose in [^{14}C-1]-glucose-infused rats). All data are expressed as means $\pm$ SEM. **significantly different from total (recycled) ^{14}C-glucose d.p.m. in soleus glycogen; *significantly different from rest, ($P < 0.05$). $n = 6$ for all bars except $NaH^{14}CO_3$-infused exercised rats where $n = 8$.

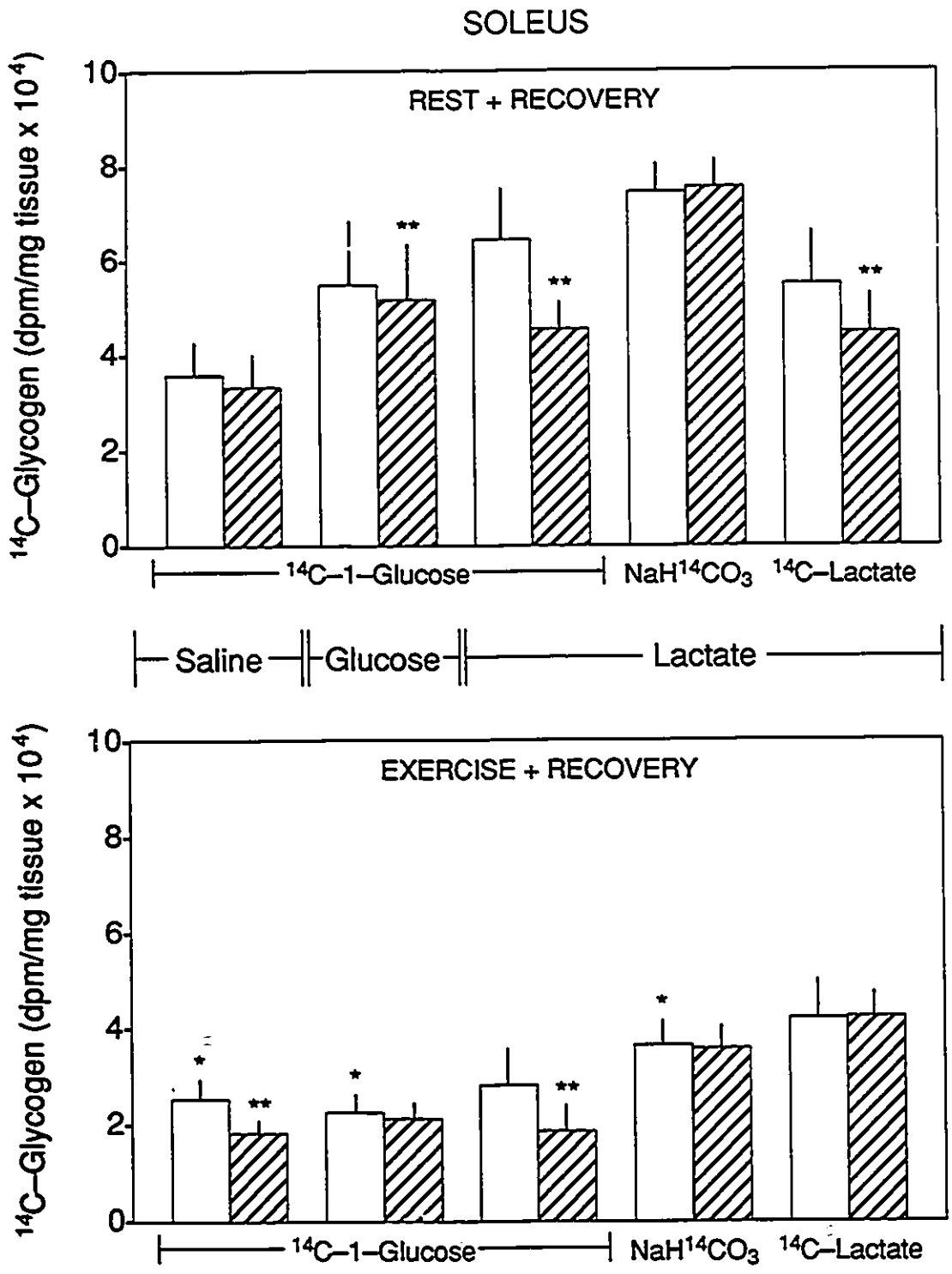

Figure 4.5: ^{14}C–Glycogen in soleus after either saline, glucose or lactic acid infusion during recovery.

Figure 4.6: Glycogen in soleus after either saline, glucose or lactic acid infusion during recovery.
Soleus glycogen was assessed in rats following recovery from 4 hours of exercise (exercise + recovery, lower graph) or rest (rest + recovery, upper graph). Rats infused with [^{3}H-3]- and [^{14}C-1]-glucose were also infused with either saline, glucose or lactic acid during the three-hour recovery period. $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats were infused with lactic acid during the recovery period. Open bars are total soleus glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis. All data are expressed as means $\pm$ SEM. For the same glycogen parameters: *significantly different from rest, $^{+}$significantly different from saline infusion and $^{++}$significantly different from glucose infusion (for [^{14}C-1]-glucose infusions only), ($P < 0.05$). $n = 6$ for all bars except $NaH^{14}CO_3$-infused exercised rats where $n = 8$.

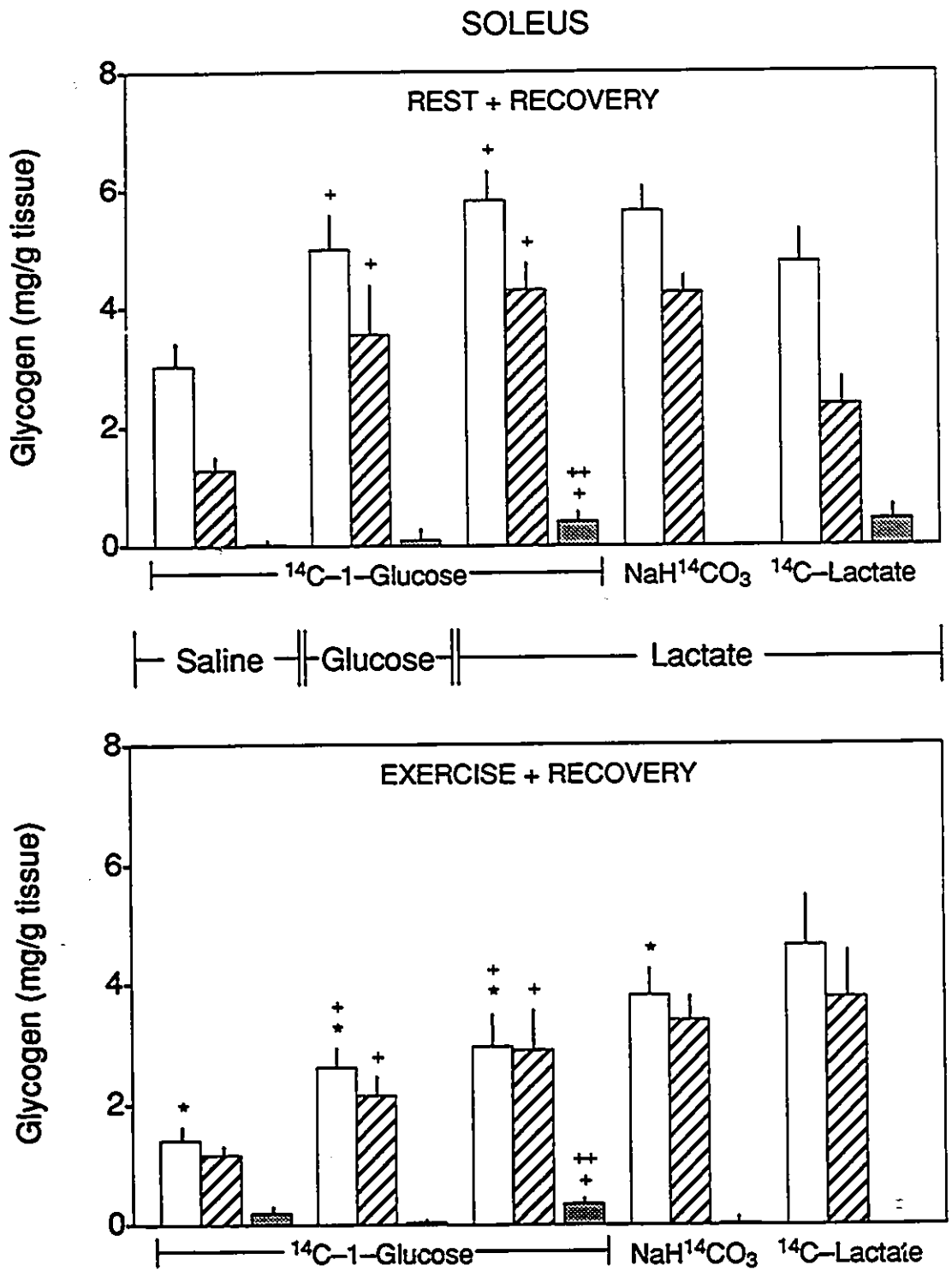

Figure 4.6: Glycogen in soleus after either saline, glucose or lactic acid infusion during recovery.

^{14}C-Glycogen in Soleus (Figure 4.5)

Rest (Upper Graph). The relatively high incorporations of ^{14}C-label from each tracer into glycogen again reflect the high turnover of glycogen at rest in the soleus muscle, with or without glucose or lactate loads. At rest, total recycled ^{14}C-glucose incorporation into glycogen in the soleus in [^{14}C-1]-glucose-infused rats was not statistically significantly increased with either glucose or lactic acid infusions. Recycled ^{14}C-glucose incorporation into glycogen was not different in glucose- and lactate-loaded rats. Although there was no difference between total recycled and direct recycled ^{14}C-glucose incorporation into glycogen in saline-infused rats, statistically, the differences were significant in both glucose- and lactic acid-infused animals ($P < 0.05$). Likewise, total and direct ^{14}C-glycogen in [^{14}C-U]-lactate-infused rats were statistically significantly different ($P < 0.05$) which may suggest some glyconeogenic activity in the muscle.

Postexercise Recovery (Lower Graph). Prior exercise decreased ^{14}C incorporation into soleus glycogen. Although total (recycled) ^{14}C-glycogen appeared lower than in rested controls in all trials, only those of saline- and glucose-infused rats ([^{14}C-1]-glucose) and those infused with $NaH^{14}CO_3$ and lactic acid were statistically lower than those at rest ($P < 0.05$). ^{14}C-glycogen arising from the direct uptake of recycled ^{14}C-glucose in plasma was statistically significantly lower than total recycled ^{14}C-glucose in glycogen in rats infused with [^{14}C-1]-glucose and either saline or lactic acid ($P < 0.05$), indicative of the possibility of some gluconeogenic glycogen synthesis in the

muscle. No glyconeogenic activity, however, was apparent in rats infused with glucose or those infused with both tracer and cold lactate. There were no differences between total recycled ^{14}C-glucose in glycogen in glucose- and lactate-loaded animals.

Glycogen Concentrations in Soleus (Figure 4.6)

Rest (Upper Graph). Both lactic acid and glucose infusions increased glycogen synthesis and total glycogen concentrations in the soleus muscle in rested rats. The amount of glycogen synthesized from glucose (direct route) increased from 1.29 $\pm$ 0.21 mg/g muscle in saline-infused rats to 3.55 $\pm$ 0.85 mg/g ($P < 0.05$) and 4.30 $\pm$ 0.43 mg/g ($P < 0.05$) with glucose and lactic acid infusions, respectively. Likewise, total glycogen concentrations in soleus in rats infused with glucose (4.98 $\pm$ 0.59 mg/g) or lactic acid (5.82 $\pm$ 0.51 mg/g) were significantly higher than those infused with saline (3.03 $\pm$ 0.38 mg/g) ($P < 0.05$). The uncorrected indices of glyconeogenesis as traced by [^{14}C-U]-lactate and [^{14}C-1]-glucose were 0.44 $\pm$ 0.21 mg/g and 0.41 $\pm$ 0.15 mg/g, respectively, the latter being significantly higher as compared to both saline- and glucose-infused rats ($P < 0.05$).

Postexercise Recovery (Lower Graph). In the soleus, total glycogen concentration was the only parameter significantly affected by prior exercise. Glycogen concentrations with saline, glucose and lactic acid infusions during recovery were significantly reduced (halved) in exercised as compared to rested rats ($P < 0.05$) (with the exception of [^{14}C-U]-lactate trials, presumably owing to individual glycogen var-

iations). The relative effects of glucose and lactic acid infusions on total, direct and gluconeogenic glycogen were similar to those described in rested animals. Total glycogen in the soleus was significantly higher with glucose (2.62 $\pm$ 0.30 mg/g) and lactate (2.96 $\pm$ 0.54 mg/g) loads as compared to saline-infused rats (1.41 $\pm$ 0.19 mg/g) ($P < 0.05$). Glycogen synthesized by the direct route was also higher in glucose- and lactic acid-infused rats (2.15 $\pm$ 0.33 and 2.90 $\pm$ 0.69 mg/g, respectively) than in those infused with saline (1.17 $\pm$ 0.13 mg/g) ($P < 0.05$). Total glycogen and that synthesized by the direct route in lactate-infused rats were not different from those in glucose-infused animals (at rest or during postexercise recovery). The uncorrected glyconeogenic index in the soleus in lactate-infused ([^{14}C-1]-glucose) rats recovering from prolonged swimming (0.35 $\pm$ 0.09 mg/g) was statistically significantly higher than those of saline- and glucose-infused animals ($P < 0.05$), but was virtually equal to that at rest. Because all of the ^{14}C-glycogen could be accounted for by the direct uptake of ^{14}C-glucose from plasma in [^{14}C-U]-lactate-infused rats (Figure 4.5), there was no recorded value of the index of gluconeogenic glycogen formation (Figure 4.6, lower graph).

Figure 4.7: ^{14}C-Glycogen in white gastrocnemius after either saline, glucose or lactic acid infusion during recovery.
^{14}C-glucose in white gastrocnemius glycogen was determined in rats following recovery from 4 hours of exercise (exercise + recovery, lower graph) or rest (rest + recovery, upper graph). Rats infused with [^{3}H-3]- and [^{14}C-1]-glucose were also infused with either saline, glucose or lactic acid during the three-hour recovery period. $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats were infused with lactic acid during the recovery period. Open bars are total ^{14}C-glucose d.p.m. in white gastrocnemius glycogen (total recycled ^{14}C-glucose d.p.m. in the cases of [^{14}C-1]-glucose infusion); striped bars are ^{14}C-glucose d.p.m. in white gastrocnemius glycogen accounted for by the direct uptake of plasma ^{14}C-glucose (recycled ^{14}C-glucose in [^{14}C-1]-glucose-infused rats). All data are expressed as means $\pm$ SEM. **significantly different from total (recycled) ^{14}C-glucose d.p.m. in white gastrocnemius glycogen; *significantly different from rest ($P < 0.05$). $n = 6$ for all bars except $NaH^{14}CO_3$-infused exercised rats where $n = 8$.

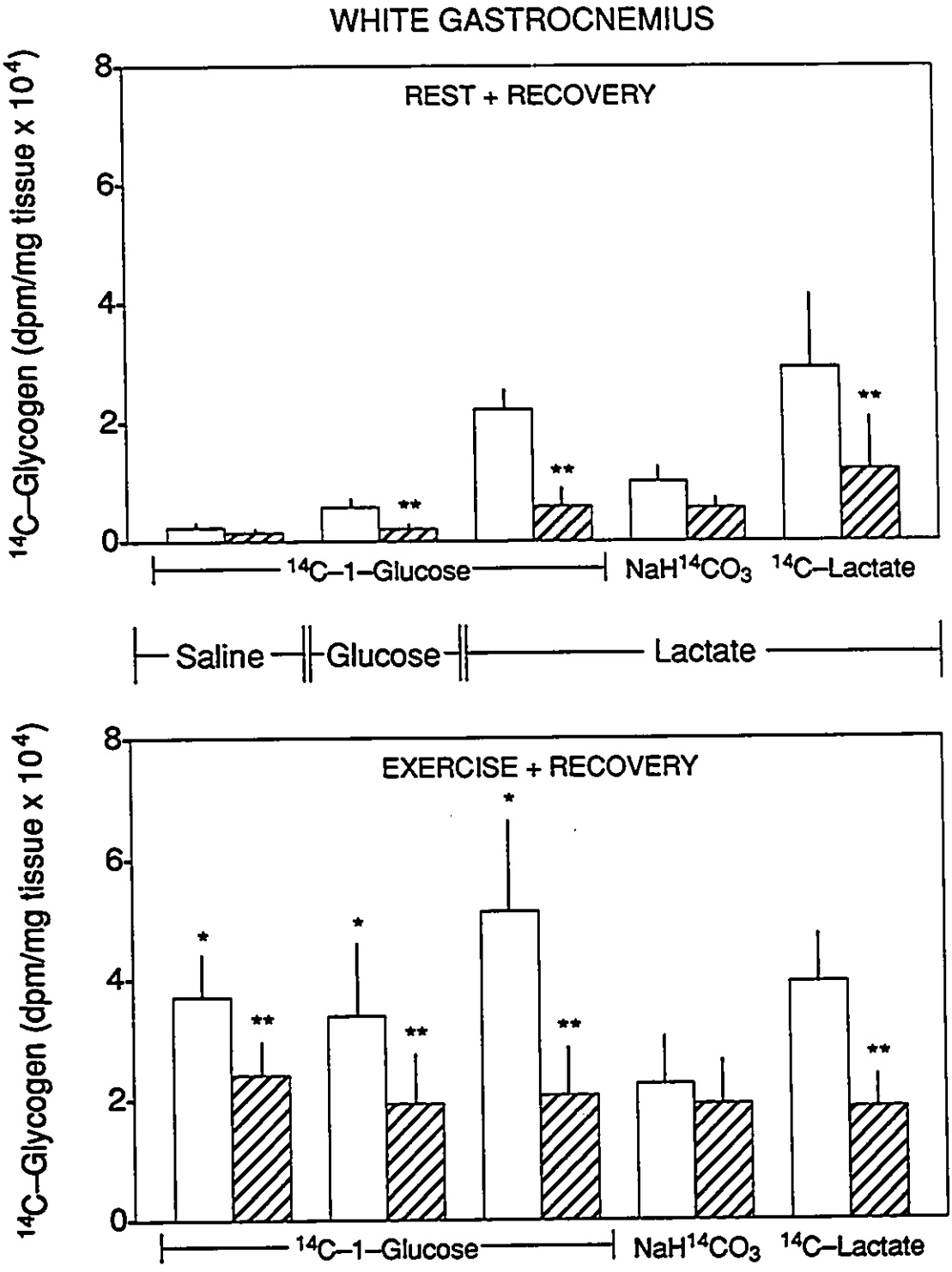

Figure 4.7: ^{14}C–Glycogen in white gastrocnemius after either saline, glucose or lactic acid infusion during recovery.

Figure 4.8: Glycogen in white gastrocnemius after either saline, glucose or lactic acid infusion during recovery.
White gastrocnemius glycogen was assessed in rats following recovery from 4 hours of exercise (exercise + recovery, lower graph) or rest (rest + recovery, upper graph). Rats infused with [^{3}H-3]- and [^{14}C-1]-glucose were also infused with either saline, glucose or lactic acid during the three-hour recovery period. $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats were infused with lactic acid during the recovery period. Open bars are total white gastrocnemius glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis. All data are expressed as means $\pm$ SEM. For the same glycogen parameters: *significantly different from rest, $^{+}$significantly different from saline infusion and $^{++}$significantly different from glucose infusion (for [^{14}C-1]-glucose infusions only), ($P < 0.05$). $n = 6$ for all bars except $NaH^{14}CO_3$-infused exercised rats where $n = 8$.

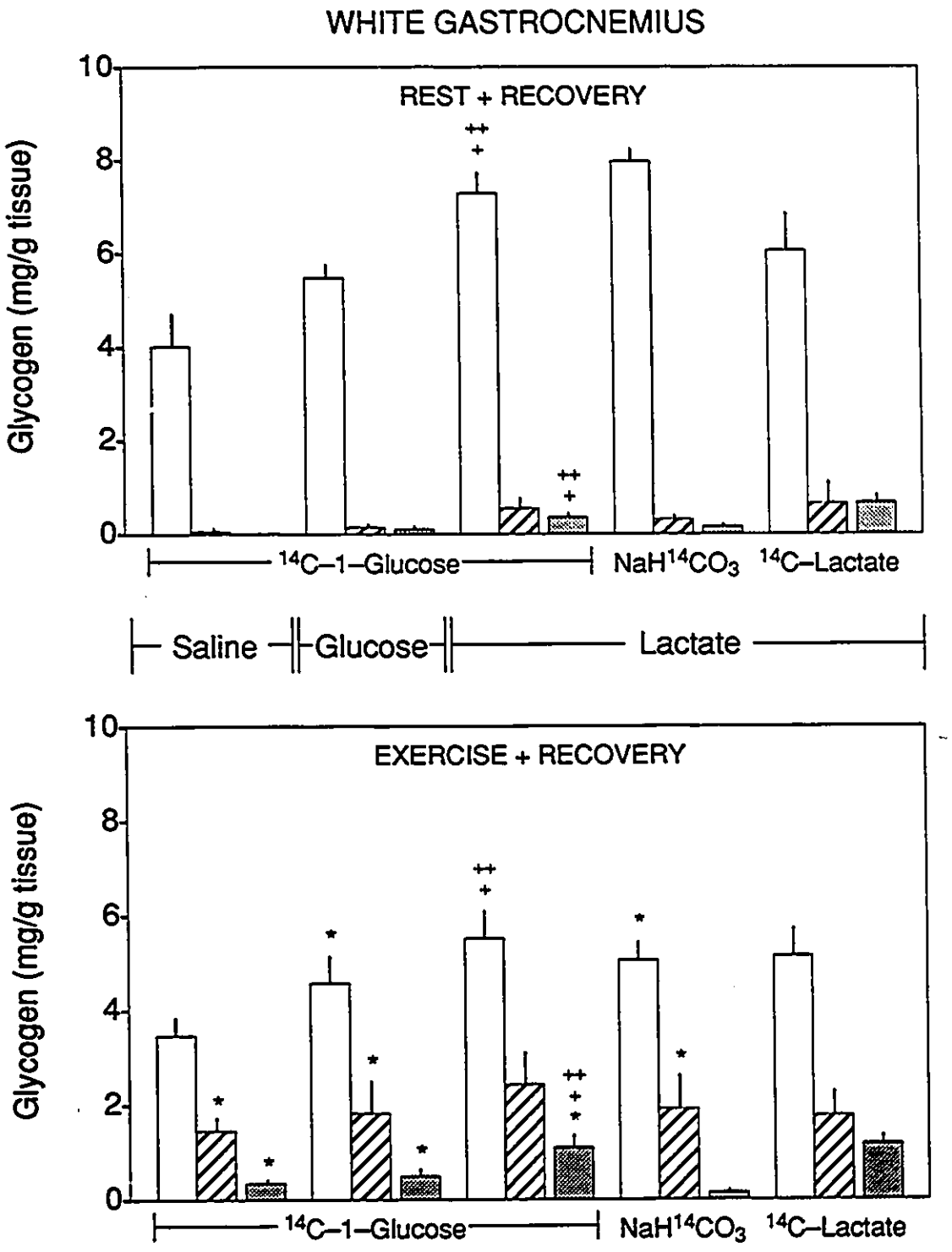

Figure 4.8: Glycogen in white gastrocnemius after either saline, glucose or lactic acid infusion during recovery.

^{14}C-Glycogen in White Gastrocnemius (Figure 4.7)

Rest (Upper Graph). With or without exogenous glucose or lactic acid infusions, incorporations of recycled ^{14}C-glucose were very low in the white gastrocnemius as compared to those in the soleus muscle (Figure 4.5), reflecting the very low rates of glycogen turnover at rest in the muscle. Total recycled ^{14}C-glucose incorporation into glycogen in the white gastrocnemius was not statistically significantly increased with the infusion of lactic acid. There were, however, statistically significant differences between total and direct (recycled) ^{14}C-glycogen with both glucose and lactic acid infusions ($P < 0.05$), indicative of glyconeogenic activity in the muscle.

Postexercise Recovery (Lower Graph). Prior exercise stimulated glycogen synthesis in the white gastrocnemius as denoted by the significantly increased total recycled ^{14}C-glucose incorporation into glycogen in rats infused with [^{14}C-1]-glucose and either saline, glucose or lactic acid as compared to rested controls ($P < 0.05$). Only about half of the ^{14}C in glycogen could be accounted for by the direct uptake of labelled plasma glucose in both [^{14}C-1]-glucose and [^{14}C-U]-lactate series, suggesting that glyconeogenesis was a significant contributor to glycogen, especially with exogenous lactate loading during postexercise recovery.

Glycogen Concentrations in White Gastrocnemius (Figure 4.8)

Rest (Upper Graph). Lactic acid infusion significantly increased total glycogen concentration (to 7.29 ± 0.47 mg/g) in the white gastrocnemius over those infused with saline (4.02 ± 0.75 mg/g, $P < 0.05$) or glucose (5.48 ± 0.26 mg/g, $P < 0.05$) in the [^{14}C-1]-glucose series. With lactic acid infusion, the increase in direct glycogen synthesis from glucose (0.55 ± 0.25 mg/g) was not statistically significant as compared to direct synthesis in saline (0.06 ± 0.02 mg/g) and glucose (0.15 ± 0.05 mg/g) trials. The glyconeogenic index in these lactate-loaded rats (0.36 ± 0.05 mg/g) was significantly higher than those of rats infused with saline (0.02 ± 0.01 mg/g, $P < 0.05$) and in animals which received a glucose load (0.11 ± 0.02 mg/g, $P < 0.05$). The uncorrected index of glyconeogenesis as detected using [^{14}C-U]-lactate in rats infused with lactic acid (0.68 ± 0.18 mg/g) matched glycogen made via the direct route (0.66 ± 0.46 mg/g).

Postexercise Recovery (Lower Graph). Prior prolonged swimming affected glycogen concentrations and the amounts of glycogen synthesized by both direct and indirect routes during recovery in the white gastrocnemius. Total glycogen concentrations in [^{14}C-1]-glucose-, glucose-infused rats (4.57 ± 0.58 mg/g) and in animals infused with $NaH^{14}CO_3$ and lactic acid (5.05 ± 0.42 mg/g) were significantly lower following three hours of postexercise recovery as compared to rested controls ($P < 0.05$). In rats infused with lactic acid during postexercise recovery ([^{14}C-1]-glucose series), total glycogen (5.52 ± 0.60 mg/g) was significantly higher than glycogen concentrations in

rats which received postexercise saline (3.47 $\pm$ 0.38 mg/g, $P < 0.05$) or glucose infusions (4.57 $\pm$ 0.58 mg/g, $P < 0.05$). Although direct glycogen synthesis was significantly increased in exercised rats during recovery over those of rested controls ($P < 0.05$), the amounts of glycogen formed from glucose were not different during postexercise saline (1.46 $\pm$ 0.27 mg/g), glucose (1.83 $\pm$ 0.70 mg/g) and lactic acid infusions (2.44 $\pm$ 0.72, 1.92 $\pm$ 0.76 and 1.78 $\pm$ 0.57 mg/g in [^{14}C-1]-glucose, $NaH^{14}CO_3$ and [^{14}C-U]-lactate trials, respectively). The indices of glyconeogenesis (as traced by recycled ^{14}C-glucose) were higher in exercised rats than in nonexercised controls with postexercise saline, glucose and lactate loads ($P < 0.05$). In the [^{14}C-1]-glucose series, the uncorrected glyconeogenic index in lactic acid-infused rats (1.10 $\pm$ 0.25 mg/g) was also significantly higher than those of animals infused with saline (0.35 $\pm$ 0.04 mg/g, $P < 0.05$) or glucose (0.50 $\pm$ 0.12 mg/g, $P < 0.05$) and corresponded precisely with the glyconeogenic index in rats infused with both tracer and cold lactate (1.18 $\pm$ 0.21 mg/g).

Figure 4.9: ^{14}C-Glycogen in red gastrocnemius after either saline, glucose or lactic acid infusion during recovery.
^{14}C-glucose in red gastrocnemius glycogen was determined in rats following recovery from 4 hours of exercise (exercise + recovery, lower graph) or rest (rest + recovery, upper graph). Rats infused with [^{3}H-3]- and [^{14}C-1]-glucose were also infused with either saline, glucose or lactic acid during the three-hour recovery period. $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats were infused with lactic acid during the recovery period. Open bars are total ^{14}C-glucose d.p.m. in red gastrocnemius glycogen (total recycled ^{14}C-glucose d.p.m. in the cases of [^{14}C-1]-glucose infusion); striped bars are ^{14}C-glucose d.p.m. in red gastrocnemius glycogen accounted for by the direct uptake of plasma ^{14}C-glucose (recycled ^{14}C-glucose in [^{14}C-1]-glucose-infused rats). All data are expressed as means $\pm$ SEM. **significantly different from total (recycled) ^{14}C-glucose d.p.m. in red gastrocnemius glycogen; *significantly different from rest ($P < 0.05$). $n = 6$ for all bars except $NaH^{14}CO_3$-infused exercised rats where $n = 8$.

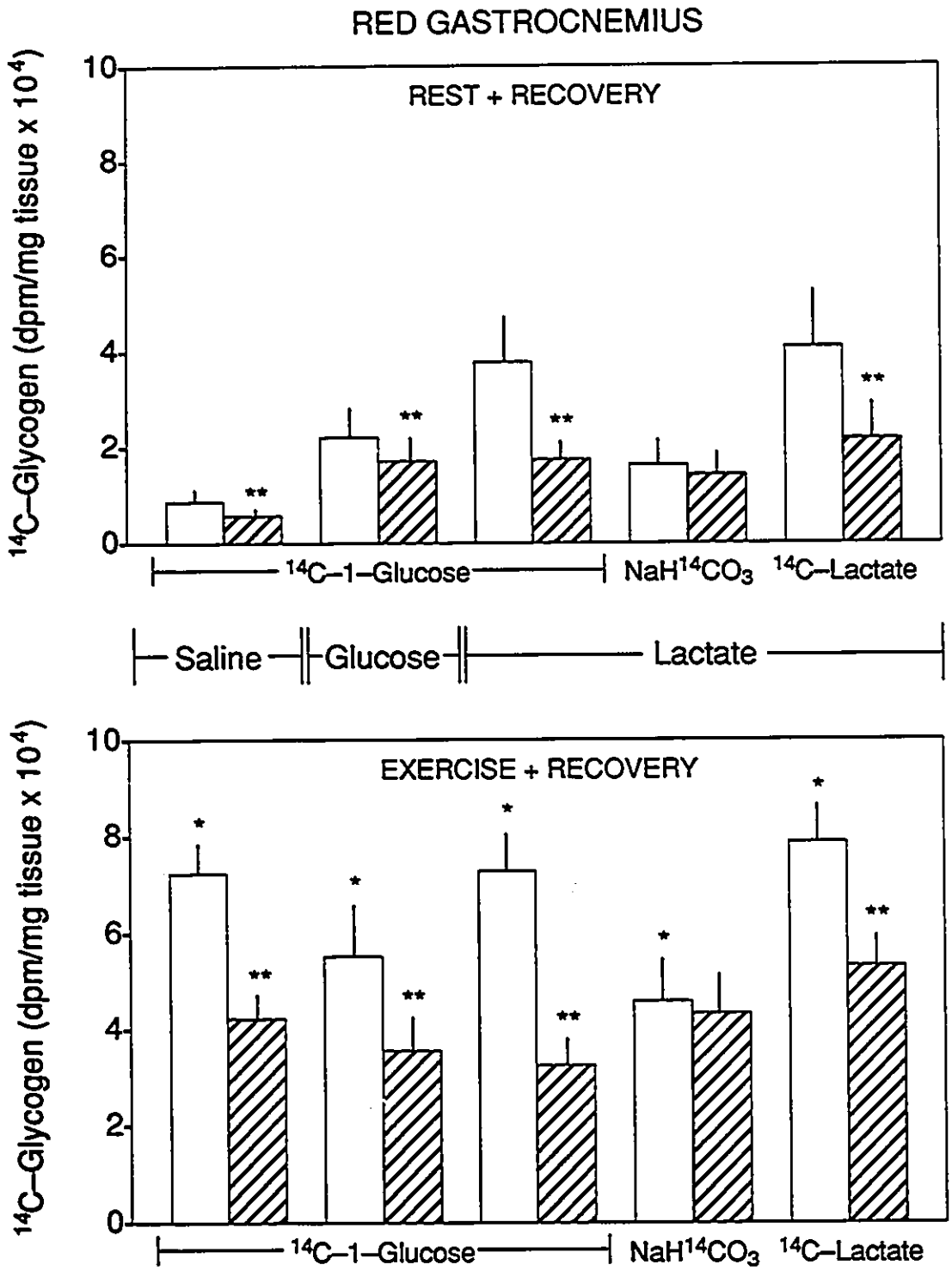

Figure 4.9: ^{14}C–Glycogen in red gastrocnemius after either saline, glucose or lactic acid infusion during recovery.

Figure 4.10: Glycogen in red gastrocnemius after either saline, glucose or lactic acid infusion during recovery.
Red gastrocnemius glycogen was assessed in rats following recovery from 4 hours of exercise (exercise + recovery, lower graph) or rest (rest + recovery, upper graph). Rats infused with [^{3}H-3]- and [^{14}C-1]-glucose were also infused with either saline, glucose or lactic acid during the three-hour recovery period. $NaH^{14}CO_3$- and [^{14}C-U]-lactate-infused rats were infused with lactic acid during the recovery period. Open bars are total red gastrocnemius glycogen concentrations; striped bars are glycogen synthesized directly from glucose; and filled bars are indices of glyconeogenesis. All data are expressed as means $\pm$ SEM. For the same glycogen parameters: *significantly different from rest, $^{+}$significantly different from saline infusion and $^{++}$significantly different from glucose infusion (for [^{14}C-1]-glucose infusions only), ($P < 0.05$). $n = 6$ for all bars except $NaH^{14}CO_3$-infused exercised rats where $n = 8$.

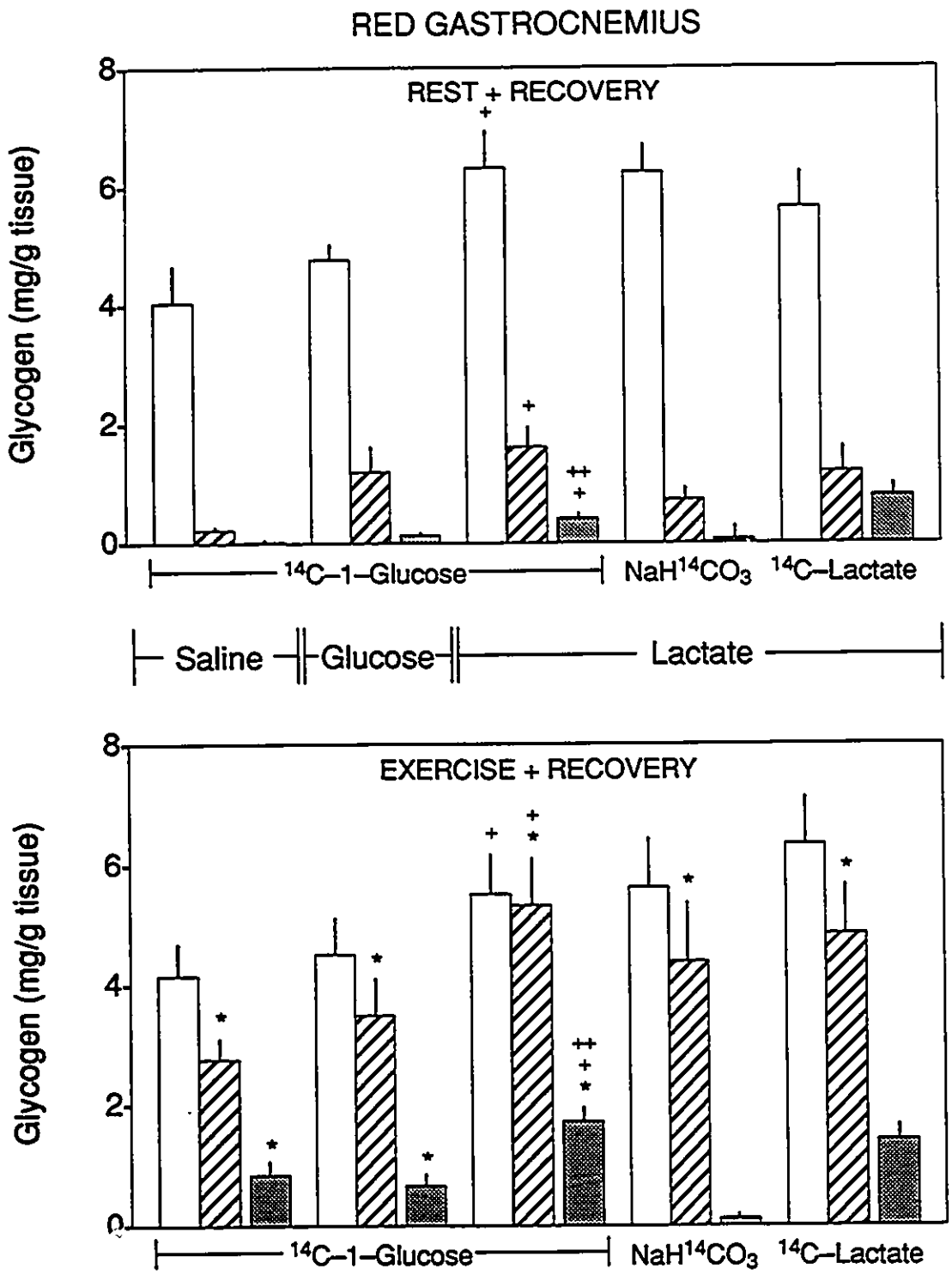

Figure 4.10: Glycogen in red gastrocnemius after either saline, glucose or lactic acid infusion during recovery.

^{14}C-Glycogen in Red Gastrocnemius (Figure 4.9)

Rest (Upper Graph). The very low turnover of glycogen at rest in the red gastrocnemius was not statistically significantly increased with either glucose or lactate infusions (as denoted by relative recycled ^{14}C-glucose incorporation into glycogen in [^{14}C-1]-glucose trials). With these glucose and lactic acid infusions, respectively, however, the significant differences between total and direct recycled ^{14}C-glucose in glycogen became more pronounced, suggesting load-dependent increases in glyconeogenic activity in the muscle. Similarly, only about half of the ^{14}C-d.p.m. in glycogen in rats infused with both tracer and cold lactate could be accounted for by the direct uptake of labelled plasma glucose, again indicative of significant gluconeogenic contributions to total glycogenesis.

Postexercise Recovery (Lower Graph). Prolonged swimming stimulated postexercise glycogen synthesis significantly in red gastrocnemii in all rats as indexed by the total amount of ^{14}C-glucose incorporation into glycogen ($P < 0.05$ as compared to nonexercised animals). Gluconeogenic glycogen formation also appeared to be augmented during postexercise recovery in all rats (with and without exogenous loading) as depicted by the significant amounts of ^{14}C-d.p.m. in glycogen unaccounted for by the direct uptake of labelled glucose from plasma in both [^{14}C-1]-glucose and [^{14}C-U]-lactate series.

Glycogen Concentrations in Red Gastrocnemius (Figure 4.10)

Rest (Upper Graph). Lactate loading induced significant increases in total glycogen levels as well as in the amounts of glycogen synthesized by both direct and indirect routes in the red gastrocnemius at rest. Total muscle glycogen in lactic acid-infused rats (6.32 ± 0.63 mg/g) was significantly higher than glycogen levels with saline infusion (4.05 ± 0.61 mg/g, $P < 0.05$) but not with glucose infusion (4.77 ± 0.25 mg/g). This same pattern applied to direct glycogen synthesis in saline- (0.23 ± 0.05 mg/g), glucose- (1.20 ± 0.41 mg/g) and lactic acid-infused rats (1.62 ± 0.31 mg/g, $P < 0.05$ *vesus* saline infusion). The index of glyconeogenesis in lactate-loaded rats (0.42 ± 0.11 mg/g) was significantly higher than the indices in both saline- (0.03 ± 0.04 mg/g, $P < 0.05$) and glucose-infused animals (0.14 ± 0.02 mg/g, $P < 0.05$). The uncorrected index of glyconeogenesis in [^{14}C-U]-lactate-, lactic acid-infused animals (0.79 ± 0.21 mg/g) approached the amount of glycogen formed via the direct route (1.20 ± 0.42 mg/g) in the muscle.

Postexercise Recovery (Lower Graph). Prior exercise stimulated direct and gluconeogenic glycogen synthesis in the red gastrocnemius during the recovery period with and without exogenous substrate and, thus, total muscle glycogen was not significantly different from rested controls following recovery from the glycogen-depleting prolonged swim. Postexercise lactic acid infusion resulted in a muscle glycogen level (5.52 ± 0.67 mg/g) significantly higher than that of rats which received saline during the recovery period (4.16 ± 0.51 mg/g, $P < 0.05$). Glycogen synthesized directly

from plasma glucose was significantly higher in all exercised rats infused with either saline (2.76 ± 0.35 mg/g), glucose (3.50 ± 0.61 mg/g) or lactic acid (5.33 ± 0.78, 4.40 ± 0.99 and 4.86 ± 0.79 mg/g in [^{14}C-1]-glucose, NaH$^{14}CO_3$ and [^{14}C-U]-lactate experiments, respectively) as compared to their respective rested controls ($P < 0.05$). Direct glycogen synthesis in the lactic acid-infused rats was also significantly higher than that resulting from saline infusion ($P < 0.05$). Prior exercise induced significant increases in the amounts of glycogen synthesized via the glyconeogenic route with saline (0.85 ± 0.20 mg/g) and glucose infusions (0.67 ± 0.17 mg/g), $P < 0.05$ as compared to respective rested controls. The index of gluconeogenic glycogen formation in lactic acid-infused rats (1.73 ± 0.23 mg/g) was not only significantly higher than that of rested controls ($P < 0.05$), but also as compared to the indices in both saline- and glucose-infused animals ($P < 0.05$). Glyconeogenesis as detected by [^{14}C-U]-lactate in lactic acid-infused rats (1.42 ± 0.21 mg/g) compared well to the minimal estimate determined via glucose recycling (1.73 ± 0.23 mg/g).

In exercised rats infused with lactic acid and [^{14}C-1]-glucose, the sum of the amounts of glycogen made via the direct and glyconeogenic routes is apparently greater than total glycogen concentration in the red gastrocnemius muscle. This is most likely due, again, to individual variations in the glycogen concentrations in the rats and to the limitations of the methodology. While total glycogen and that formed via the direct route should, theoretically, be consistent with those in all lactate-infused rats as the determinations were made in the same way, the mean total glycogen concentration in the [^{14}C-1]-glucose series is lower than and direct glycogen synthesis

is higher than those in both the [^{14}C-U]-lactate and $NaH^{14}CO_3$ series. In addition, the amounts of glycogen synthesized by the direct and glyconeogenic routes were calculated (differently) from several values whereas total glycogen represents one measurement. It should be noted as well that total glycogen concentration is simply the amount of glycogen in the muscle and does not reflect new synthesis as do the values for both direct and gluconeogenic glycogen. Thus, comparing the higher direct synthesis (with its accompanying error) to the lower total glycogen concentration (with its individual variation) may give the false impression that all of the glycogen was made via the direct route leaving little room for any gluconeogenic contributions. However, the glyconeogenic index supplies a more precise and independent estimate of this particular process. Interestingly, the two estimates, under the same circumstances, using different tracers, are very similar in magnitude.

Table 4.1: Gluconeogenic glycogen synthesis as indexed by recycled ^{14}C-glucose d.p.m. in tissue glycogen not arising from ^{14}C-glucose from plasma.
The total amount of recycled ^{14}C-glucose in tissue glycogen (liver = L, soleus = S, white gastrocnemius = WG and red gastrocnemius = RG) was assessed in [^{14}C-1]-glucose-infused rats following two hours of rest, four hours of swimming or rest and three hours of saline (sal), lactic acid (lac) or glucose (gluc) infusion. That which could not be accounted for by the direct uptake of recycled ^{14}C-glucose from plasma (as traced using [^{3}H-3]-glucose infusion) was used as a relative index of gluconeogenic glycogen formation. The results were expressed as the means of the differences (in ^{14}C-d.p.m.) between total and direct recycled ^{14}C-glycogen $\pm$ SEM (for n = 6 rats). *significantly different from rest, $^{+}$significantly different from saline infusion and $^{++}$significantly different from glucose infusion ($P < 0.05$). **Column D of the relative glyconeogenic indices immediately following the swim was taken from Chapter 3 data and was not used in the statistical analysis.

	Gluconeogenic Glycogen Synthesis (^{14}C-d.p.m./mg)						
	A	B	C	D**	E	F	G
	rest + sal	rest + lac	rest + gluc	swim	swim + sal	swim + lac	swim + gluc
L	12136 ± 3092	21836 ± 5869 ++	83128 ± 13572 +	4337 ± 1352	20869 ± 9040	19552 ± 3601 ++	65423 ± 6185 +
S	2502 ± 2889	19060 ± 6546 ++	3130 ± 3159	-9999 ± 5226	7089 ± 2459	9537 ± 2382 ++	-1766 ± 2994
WG	878 ± 354	16332 ± 1768 + ++	3756 ± 658	2882 ± 2186	12953 ± 1630 *	30593 ± 7423 * + ++	14587 ± 3670 *
RG	2921 ± 1350	20265 ± 5908 + ++	4993 ± 807	807 ± 5698	30223 ± 6608 *	40307 ± 3595 * + ++	19698 ± 4803 *

Table 4.1: Gluconeogenic glycogen synthesis as indexed by recycled ^{14}C-glucose d.p.m. in tissue glycogen not arising from recycled ^{14}C-glucose from plasma.

Gluconeogenic Glycogen Synthesis (Table 4.1)

The differences (in ^{14}C-d.p.m.) between total recycled ^{14}C-glucose in glycogen and that which could be accounted for by the direct uptake of recycled ^{14}C-glucose from plasma were used also used as relative indices (in conjunction with the absolute (mg/g) indices of glyconeogenesis) to assess the effects of exercise and of glucose and lactic acid infusions on glyconeogenesis in liver and muscle. (Column D of the differences between total and direct recycled ^{14}C-glycogen d.p.m. immediately following prolonged swimming was taken from Chapter 3 and has been included for the sake of qualitative comparison; no statistical analyses have been done on these values in this chapter.) Hepatic gluconeogenic glycogen synthesis was stimulated to a significant degree by glucose infusion as compared to both saline and lactic acid infusions ($P < 0.05$) in both rested and exercised animals, with exercise alone having no apparent stimulatory effect. The relatively low ^{14}C-d.p.m. in the soleus in saline-infused rested and exercised rats were both significantly increased with lactic acid infusions ($P < 0.05$). Prior exercise significantly stimulated gluconeogenic glycogen synthesis in both white and red gastrocnemii in saline-, glucose- and lactic acid-infused rats ($P < 0.05$ *versus* their respective rested control values). Glyconeogenesis in white and red gastrocnemii in both rested and exercised animals was significantly increased with lactic acid infusion as compared to that resulting from saline or glucose infusions in rested or exercised rats, respectively ($P < 0.05$).

DISCUSSION

Intense exercise can induce twenty-fold increases in muscle and plasma lactate concentrations (eg. refs. 3, 18) which may provide substrate for postexercise gluconeogenic repletion of muscle glycogen. Hermansen and Vaage (18) have reported plasma and muscle lactate levels of 21 mM and 26 $mmol \cdot kg^{-1}$ wet weight, respectively, immediately following maximal intermittent exercise in humans and have suggested that muscle lactate is the primary substrate for postexercise muscle glycogen repletion. Most *in vitro* (25, 37, 38) and *in vivo* (22) studies demonstrate a *need* for high lactate concentrations in perfusate or plasma, respectively, for glyconeogenesis to be induced. Our studies (Chapters 2 and 3), however, have shown that, following submaximal exercise characterized by minimal lactate accumulation, while no glyconeogenesis is detectable as traced by [^{14}C-U]-lactate (given systemically), gluconeogenic glycogen synthesis is seen primarily in fast-twitch muscles (white and red gastrocnemii) when recycled ^{14}C-glucose is used. This implies that: *1)* the enzymatic machinery for the glyconeogenic process is present in muscle as previously seen (4, 22, 25, 37, 38, 39, 40); *2)* it is operative under physiological circumstances (this being comparable to the *in vitro* results of Bonen et al. (4) demonstrating glyconeogenesis at low lactate levels); and, *3)* the substrate for glyconeogenesis appears to be that which is produced locally in the muscles since it does not equilibrate well with systemic lactate. The results in the present study extend these findings to show that, in fast-twitch muscles: *1)* in the presence of high lactate levels, and particularly in the postexercise period, gluconeogenic glycogen synthesis is

enhanced (independent of resulting changes in plasma glucose concentrations); and, *2)* the individual effects of lactate and prior exercise are additive in terms of glycogen synthesis during recovery.

THE EFFECTS OF LACTATE INFUSION ON MUSCLE GLYCOGENESIS

WHITE AND RED GASTROCNEMII

Glyconeogenesis is demonstratable in white and red gastrocnemii in the presence of elevated lactate levels in rested and exercised animals using both ^{14}C-lactate and recycled ^{14}C-glucose as markers. The provision of lactate increased both the amounts of glycogen made via the indirect route and the percent contributions made by glyconeogenesis to glycogenesis in the fast-twitch muscles in rested rats. The percent contributions of glyconeogenesis to total glycogen synthesis increased from 12% and 25% to 21% and 40% in red and white gastrocnemius, respectively, with the infusion of lactic acid into rested animals (as traced using [^{14}C-1]-glucose). In rats recovering from exercise, the amount of glycogen formed via the gluconeogenic route was enhanced with lactic acid infusion and accounted for 30-40% and 23% in the white and red muscles, respectively.

These contributions to glycogenesis are based on the uncorrected indices of glyconeogenesis as determined using both ^{14}C-lactate and recycled ^{14}C-glucose. With exogenous lactate loading, the majority of the glycogen formed via the gluconeogenic pathway would be expected to originate from the very high lactate concentrations in plasma. The two tracers, then, should give comparable results, which they do. The

variations that are seen, however small, may be accounted for by the following reasons. The glyconeogenic indices in lactate-loaded rats infused with [^{14}C-1]-glucose were calculated using the average specific activities of ^{14}C-glucose in plasma (as the precursor to ^{14}C-lactate in muscle) rather than those of plasma ^{14}C-lactate (as in all other determinations) as it was expected that plasma lactate specific activity would be significantly diluted by the high concentrations of lactate in the blood and would not be reflective of muscle lactate specific activity. Secondly, the percent gluconeogenic contributions were calculated as the indices of glyconeogenesis divided by the sum of direct synthesis and the indices of glyconeogenesis for each group of rats; variations (or inconsistencies) in direct synthesis, then, would affect total glycogenesis and, thus, percent gluconeogenic contributions.

All of these estimates, however, are based on the *uncorrected* indices of glyconeogenesis and may, in fact, underestimate actual amounts of glycogen formed via the indirect route by as much as 35-50% (4, 19, 25, 39). With such considerations, then, the glyconeogenic route may indeed be the predominant means of glycogen synthesis in the presence of high lactate levels.

Relative contributions by the indirect pathway to glycogenesis reported in the literature vary widely. Our minimal estimates of percent gluconeogenic glycogen formation in muscle do not approach the calculated 95% contribution reported in human muscle following maximal exercise (18) but are higher than those (also uncorrected for label dilution) reported in mouse extensor digitorum longus (9% and 16%) incubated in lactate concentrations of 10 and 20 mM, respectively (4). The

discrepancies may be rooted in species differences in relative activities of gluconeogenic enzymes in the different muscles (10) and to experimental design (perfusion *versus in vivo* analysis). Johnson and Bagby (22), on the other hand, have reported similar glyconeogenic activity in rat muscle accounting for approximately 50% of total glycogen formation; however, glycogeneogenesis was detected exclusively in white gastrocnemius and only in the presence of elevated lactate levels.

Tables 4.2 and 4.3 (pp. 179-180) illustrate, in a quasi-quantitative manner, the relative individual effects of exercise, fasted recovery, lactate and the combination of high lactate and prior exercise on gluconeogenic and direct glycogen synthesis in the muscles, respectively. While lactate alone induced gluconeogenic glycogen formation in the gastrocnemius muscles, exercise plus postexercise recovery seems to be a more potent stimulus for both direct and indirect glycogen synthesis in the gastrocnemii. In the gastrocnemius muscles, the stimulatory effects of high lactate concentrations during postexercise recovery on direct and gluconeogenic glycogenesis were slightly greater than the sums of the individual effects of exercise, postexercise recovery and lactate, suggesting that the effects of lactate and exercise on glycogenesis are additive. An analogous analysis using the differences between total and direct recycled ^{14}C-glycogen (Table 4.1) in the various cases confirm this potential additive relationship of the effects of exercise and lactate on postexercise glyconeogenesis.

	Gluconeogenic Glycogen Synthesis (mg/g tissue)			
	LIVER	SOLEUS	WHITE GASTROC	RED GASTROC
Exercise	- -	- -	0.05	- -
Recovery	0.65	0.20	0.30	0.85
Lactate	0.85*	0.38	0.34	0.39
Glucose	2.10	0.06	0.09	0.11
Exercise + Lactate	2.60*	0.35	1.05	1.73
Exercise + Glucose	2.24	0.05	0.45	0.67

Table 4.2: Effects of various parameters on gluconeogenic glycogen synthesis in liver and muscle.
Indices of gluconeogenic glycogen synthesis were calculated in rats following 4 hours of swimming or rest (Chapter 3), as well as following three-hour infusions of either saline, lactate or glucose during recovery from exercise or rest. These uncorrected indices in the appropriate groups of rats (in italics) were used to calculate the relative individual stimulatory effects of exercise, postexercise recovery, lactate, glucose and substrate loading following exercise on glyconeogenesis (each in quotations) as specified below:

"Exercise" = *exercise (no recovery) - rest (no recovery)* (from Chapter 3)
"Recovery" = *saline infusion (postexercise)* - "Exercise"
"Lactate" = *lactate infusion (rest) - saline infusion (rest)*
"Glucose" = *glucose infusion (rest) - saline infusion (rest)*
"Exercise + Lactate" = *lactate infusion (postexercise)* - "Exercise"
"Exercise + Glucose" = *glucose infusion (postexercise)* - "Exercise"

*Effects based on glyconeogenic indices from lactate-loaded $NaH^{14}CO_3$ trials.

	Direct Glycogen Synthesis (mg/g tissue)			
	LIVER	SOLEUS	WHITE GASTROC	RED GASTROC
Exercise	- -	- -	0.68	1.40
Recovery	0.29	0.02	0.69	0.93
Lactate	0.45	3.01	0.49	1.39
Glucose	2.78	2.26	0.09	0.97
Exercise + Lactate	1.37	1.75	2.62	3.70
Exercise + Glucose	3.29	1.00	1.06	1.67

Table 4.3: Effects of various parameters on direct glycogen synthesis in liver and muscle.
Direct glycogen synthesis was calculated in rats following 4 hours of swimming or rest (Chapter 3), as well as following three-hour infusions of either saline, lactate or glucose during recovery from exercise or rest. Glycogen formed by the direct uptake of plasma glucose in the appropriate groups of rats (italics) was used to calculate the relative individual stimulatory effects of exercise, postexercise recovery, lactate, glucose and substrate loading following exercise on direct glycogenesis as specified below:

"Exercise" = *exercise (no recovery) - rest (no recovery)* (from Chapter 3)
"Recovery" = *saline infusion (postexercise)* - "Exercise"
"Lactate" = *lactate infusion (rest) - saline infusion (rest)*
"Glucose" = *glucose infusion (rest) - saline infusion (rest)*
"Exercise + Lactate" = *lactate infusion (postexercise)* - "Exercise"
"Exercise + Glucose" = *glucose infusion (postexercise)* - "Exercise"

SOLEUS

There is some indication that glyconeogenesis may occur in the soleus muscle in the presence of high lactate levels. The contributions to total glycogen synthesis, however, are small (9% and 11% in lactate-loaded rested and exercised rats as traced by recycled ^{14}C-glucose) and not completely consistent since ^{14}C-lactate indicates no glyconeogenic activity when lactate is given following exercise. These estimates, however, are higher than the 1.1% and 1.6% glyconeogenic contributions reported in mouse soleus incubated in 10 and 20 mM lactate (4). If any glyconeogenesis does occur, it appears to be independent of exercise since the three indices (two in rested and one in exercised rats) are very nearly equal (approximately 0.4 mg/g). That the soleus muscle responds more to the availability of substrate rather than to prior exercise to synthesize glycogen both indirectly and directly is evident in Tables 4.2 and 4.3, respectively, which show that the combined effects of exercise plus lactate are equivalent (glyconeogenesis) or even less than (direct route) those of the individual effects of lactate alone. As discussed in Chapter 3, any glyconeogenesis that might occur in the soleus may be due to the FOG fibers (15%) in this muscle (1, 2) which apparently have higher glyconeogenic capacities (25). Finally, despite any glycogenic stimulation by lactate during recovery, there is still postexercise glycogen *depletion* relative to rest, consistent with previous results (Chapters 2 and 3).

THE EFFECTS OF GLUCOSE INFUSION ON MUSCLE GLYCOGENESIS

WHITE AND RED GASTROCNEMII

Glucose had little stimulatory effect on both gluconeogenic and direct glycogen synthesis in the gastrocnemius muscles at rest or following exercise (above that which would be expected in a fasted postexercise period). Glucose, therefore, does not seem to be the principal substrate (direct or indirect) for glycogen synthesis, whereas lactate seems to increase direct glycogenesis (additively with exercise) as well as adding to glyconeogenesis. It has been shown in the liver that lactate appears to have a regulatory effect on both direct and gluconeogenic production of glycogen (42); similar mechanisms may be operating in the gastrocnemius.

SOLEUS

In contrast to white and red gastrocnemii, glucose infusion appears to provide equivalent substrate and stimulus to glycogenesis (direct) as that induced by lactate infusion (Table 4.3), without stimulating any glyconeogenic activity (Table 4.2). The effects of prior exercise appear unimportant in the stimulation of direct glycogen synthesis by glucose during recovery, again supporting the response by soleus to substrate rather than to prior swimming.

"VALIDATION"

In all of the above discussion, it is implicit that the method (and results on glyconeogenesis) are somehow "validated" by the $NaH^{14}CO_3$ data since these numbers are consistently very close to zero (and not always positive) which they are supposed to be, as discussed in Chapter 2. This lends some credence to one of the main assumptions underlying this work - that the formation of glycogen during recovery is linear. Indeed, in exercised rats recovering for only one hour in a fasted state (n = 3, preliminary data not presented), direct glycogen synthesis was approximately 1.2 and 1.8 mg/g in the white and red gastrocnemii, respectively; when plotted on a time scale with the amounts of glycogen synthesized immediately following exercise and following three hours of postexercise fasted recovery, the relationship is linear. Interestingly, this may be less true for soleus as previously discussed in Chapter 3 (using the same approach). In the liver, $NaH^{14}CO_3$ and [^{14}C-U]-lactate gave similar indices of gluconeogenic glycogen formation in lactate-loaded rested and exercised rats, while those estimated using recycled ^{14}C-glucose were expectedly lower as they were calculated using plasma ^{14}C-glucose specific activity rather than that of plasma lactate.

LIVER

The results of this study not only corroborate the many previous findings that during refeeding following fasting, glyconeogenesis is the predominant route of hepatic glycogenesis (14, 22, 23, 24, 26, 28, 29, 32, 33, 36, 42), but further suggest

a synergistic relationship between lactate and exercise on the promotion of both direct and indirect synthesis. While prior exercise and lactate each provide some (relatively minor) stimulation of both direct and indirect hepatic glycogen synthesis, glucose appears to be the most potent stimulus for liver glycogenesis (Tables 4.2 and 4.3); the combined influences of exercise plus lactate on glycogenesis, however, not only approach those of glucose, but are also nearly double the sums of the individual effects of each lactate and exercise on glyconeogenesis and direct synthesis, with the latter being the more affected.

This stimulation of glyconeogenesis by lactate and exercise is likely due to the availability of additional substrate. This synergistic effect on direct glycogen synthesis may be via increased glucose levels induced by lactate following exercise; however, if the stimulation of direct synthesis is mediated via glucose, then the combined effects of lactate plus exercise should be higher, rather than approximately half of that induced by glucose loading (see Table 4.3). Perhaps more significantly, this synergism may be related to the potential regulatory role of lactate in direct glycogen synthesis as has been previously suggested (eg. ref. 42).

REFERENCES

1. Ariano, M. A., R. B. Armstrong, and V. R. Edgerton. Hindlimb muscle fiber populations of five mammals. J. Histochem. Cytochem. 21(1): 51-55, 1973.

2. Armstrong, R. B., and R. O. Phelps. Muscle fiber type composition of the rat hindlimb. Am. J. Anat. 171: 259-272, 1984.

3. Åstrand, P. -O., E. Hultman, A. Juhlin-Dannfelt, and G. Reynolds. Disposal of lactate during and after strenuous exercise in humans. J. Appl. Physiol. 61(1): 338-343, 1986.

4. Bonen, A., J. C. McDermott, and M. H. Tan. Glycogenesis and glyconeogenesis in skeletal muscle: effects of pH and hormones. Am. J. Physiol. 258 (Endocrinol. Metab. 21): E693-E700, 1990.

5. Brooks, G. A. The lactate shuttle during exercise and recovery. Med. Sci. Sports Exerc. 18(3): 360-368, 1986.

6. Brooks, G. A., and G. A. Gaesser. End points of lactate and glucose metabolism after exhausting exercise. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(6): 1057-1069, 1980.

7. Cohen, R. D., and H. F. Woods. Lactic acidosis revisited. Diabetes 32 181-191, 1983.

8. Conlee, R. K., R. C. Hickson, W. W. Winder, J. M. Hagberg, and J. O. Holloszy. Regulation of glycogen resynthesis in muscles of rats following exercise. Am. J. Physiol. 35(3): R145-R150, 1978.

9. Connett, R. J. Glyconeogenesis from lactate in frog striated muscle. Am J. Physiol. 237(5): C231-C236, 1979.

10. Crabtree, B., S. J. Higgins, and E. A. Newsholme. The activities of pyruvate carboxylase, phosphoenolpyruvate carboxylase and fructose diphosphatase in muscles from vertebrates and invertebrates. Biochem. J. 130: 391-396, 1972.

11. Dyson, R. D., J. M. Cardenas, and R. J. Barsotti. The reversibility of skeletal muscle pyruvate kinase and an assessment of its capacity to support glyconeogenesis. J. Biol. Chem. 250(9): 3316-3321, 1975.

12. Favier, R. J., H. E. Koubi, M. H. Mayet, B. Semporé, B. Simi, and R. Flandrois. Effects of gluconeogenic precursor flux alterations on glycogen resynthesis after prolonged exercise. J. Appl. Physiol. 63(5): 1733-1738, 1987.

13. Fell, R. D., J. A. McLane, W. W. Winder, and J. O. Holloszy. Preferential resynthesis of muscle glycogen in fasting rats after exhausting exercise. Am. J. Physiol. 238 (Regulatory Integrative Comp. Physiol. 7): R238-R332, 1980.

14. Foster, D. W. From glycogen to ketones - and back. Diabetes 33: 1188-1199, 1984.

15. Gaesser, G. A., and G. A. Brooks. Glycogen repletion following continuous and intermittent exercise to exhaustion. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49(4): 722-728, 1980.

16. Gleeson, T. Glycogen synthesis from lactate in skeletal muscle of the lizard *Dipsosaurus dorsalis*. J. Comp. Physiol. B. 156: 277-283, 1985.

17. Goodman, M. N., R. Dietrich, and P. Luu. Formation of gluconeogenic precursors in rat skeletal muscle during fasted-refed transition. Am. J. Physiol. 259 (Endocrinol. Metab. 22): E513-E516, 1990.

18. Hermansen, L., and O. Vaage. Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. Am J. Physiol. 233(5): E422-E429, 1977.

19. Hetenyi, G., Jr. Correction for the metabolic exchange of ^{14}C for ^{12}C atoms in the pathway of gluconeogenesis *in vivo*. Fed. Proc. 41: 104-109, 1982.

20. Hiatt, H. H., M. Goldstein, J. Laureau, and B. L. Horecker. The pathway of hexose synthesis from pyruvate in muscle. J. Biol. Chem. 231: 303-307, 1958.

21. Hutber, C. A., and A. Bonen. Glycogenesis in muscle and liver during exercise. J. Appl. Physiol. 66(6): 2811-2817, 1989.

22. Johnson, J. L., and G. J. Bagby. Gluconeogenic pathway in liver and muscle glycogen synthesis after exercise. J. Appl. Physiol. 64(4): 1591-1599, 1988.

23. Katz, J., and J. D. McGarry. The glucose paradox: is glucose a substrate for liver metabolism? J. Clin. Invest. 74: 1901-1909, 1984.

24. Lang, C. H., G. J. Bagby, H. L. Blakesley, J. L. Johnson, and J. J. Spitzer. Plasma glucose concentration determines direct *versus* indirect liver glycogen synthesis. Am. J. Physiol. 251 (Endocrinol. Metab. 14): E584-E590, 1986.

25. McLane, J. A., and J. O. Holloszy. Glycogen synthesis from lactate in the three types of skeletal muscle. J. Biol. Chem. 254: 6548-6553, 1979.

26. Mitrakou, A., R. Jones, Y. Okuda, J. Pena, N. Nurjhan, J. B. Field, and J. E. Gerich. Pathway and carbon sources for hepatic glycogen repletion in dogs. Am. J. Physiol. 260 (Endocrinol. Metab. 23): E194-E202, 1991.

27. Naylor, J. M., D. S. Kronfeld, D. E. Freeman, and D. Richardson. Hepatic and extrahepatic lactate metabolism in sheep: effects of lactate loading and pH. Am. J. Physiol. 247 (Endocrinol. Metab. 10): E747-E755, 1984.

28. Newgard, C. B., S. V. Moore, D. W. Foster, and J. D. McGarry. Efficient hepatic glycogen synthesis in refeeding rats requires continued carbon flow through the gluconeogenic pathway. J. Biol. Chem. 259: 6958-6963, 1984.

29. Newgard, C. B., L. J. Hirsch, D. W. Foster, and J. D. McGarry. Studies on the mechanism by which exogenous glucose is converted into liver glycogen in the rat. J. Biol. Chem. 258: 8046-8052, 1983.

30. Opie, L. H., and E. A. Newsholme. The activities of fructose 1,6-diphosphatase, phosphofructokinase and phosphoenolpyruvate carboxykinase in white muscle and red muscle. Biochem. J. 103: 391-399, 1967.

31. Pagliassotti, M. J., C. M. Donovan. Glycogenesis from lactate in rabbit skeletal muscle fiber types. Am. J. Physiol. 258 (Regulatory Integrative Comp. Physiol. 27): R903-R911, 1990.

32. Radziuk, J. Sources of carbon in hepatic glycogen synthesis during absorption of an oral glucose load in humans. Fed. Proc. 41:110-116, 1982.

33. Radziuk, J. Tracer methods and the metabolic disposal of a carbohydrate load in man. Diabetes/Metabolism Reviews 3(1): 231-267, 1987.

34. Reeves, R. E. The estimation of primary carbinol groups in carbohydrates. J. Amer. Chem. Soc. 63: 1476-1477, 1941.

35. Rodbard, D. Statistical estimation of the minimal detectable concentration ("sensitivity") for radioligand assays. Anal. Biochem. 90: 1-12, 1978.

36. Scofield, R. F., L. Kosugi, W. C. Shumann, K. Kumaran, and B. R. Landau. Quantitative estimation of the pathways followed in the conversion to glycogen of glucose administered to the fasted rat. J. Biol. Chem. 260: 8777-8782, 1985.

37. Shiota, M., S. Golden, and J. Katz. Lactate metabolism in the perfused rat hindlimb. Biochem. J. 222: 281-292, 1984.

38. Stevenson, R. W., D. R. Mitchell, G. K. Hendrick, R. Rainey, A. D. Cherrington, and R. T. Frizzell. Lactate as substrate for glycogen resynthesis after exercise. J. Appl. Physiol. 62(6): 2237-2240, 1987.

39. Talmadge, R. J., J. I. Scheide, and H. Silverman. Glycogen synthesis from lactate in chronically active muscle. J. Appl. Physiol. 66(5):2231-2238, 1989.

40. Talmadge, R. J., and H. Silverman. Glyconeogenic and glycogenic enzymes in chronically active and normal skeletal muscle. J. Appl. Physiol. 71(1): 182-191, 1991.

41. Watt, P. W., P. A. MacLennan, H. S. Hundal, C. M. Kuret and M. J. Rennie. L(+)-Lactate transport in perfused rat skeletal muscle: kinetic characteristics and sensitivity to pH and transport inhibitors. Biochim. Biophys. Acta 944: 213-222, 1988.

42. Zhang, Z., and J. Radziuk. Effects of lactate on pathways of glycogen formation in the perfused rat liver. Biochem. J. 280: 415-419, 1991.

SUMMARY

During prolonged submaximal swimming in rats, the rates of both glucose production (R_a) and clearance (MCR) double to meet the increased metabolic demands of the contracting muscles. Because the increases in R_a and MCR are matched, plasma glucose levels are maintained during exercise. During postexercise recovery (in a fasted state), however, while MCR approaches basal rates, the rate of glucose production remains high, resulting in a significant increase in plasma glucose levels. That there is no apparent repletion of hepatic glycogen during a fasted postexercise recovery period suggested that the maintenance of the elevated rates of glucose production is for the purpose of the preferential repletion of muscle glycogen. The extent to which this plasma glucose contributes to muscle glycogen synthesis, however, remains obscure. Whether muscle glycogen is synthesized entirely from plasma glucose or whether muscle is capable of significant glycogenesis via the indirect or glyconeogenic route during a fasted recovery period following prolonged submaximal exercise was the central question of the thesis.

Tables I and II (pp. 191-192) summarize indices of the amounts of glycogen synthesized via glyconeogenesis and from plasma glucose, respectively. As muscle glyconeogenesis apparently occurs via lactate (or pyruvate) formed locally in the muscle, glucose recycling (as traced by [^{14}C-1]-glucose) proved to be the best assessment of the contributions of glyconeogenesis to glycogen synthesis in the fasted state. With lactate-loading, however, since the primary source of the lactate was the

plasma, [^{14}C-U]-lactate infusion is probably the best estimate of gluconeogenic glycogen formation. Each tissue will be reviewed individually with respect to its metabolic responses to prolonged exercise, postexercise recovery and high lactate levels.

SOLEUS

Glycogen synthesis in the soleus, a representative of slow-twitch oxidative muscle fibers, appears to be almost entirely (>97%) from the direct uptake of plasma glucose at rest. The indices of glyconeogenesis were consistently very low at rest with each method of assessment used (see Table I). However, the turnover rate of soleus glycogen is very high at rest, due presumably to the very high rates of direct glycogenesis (Table II).

The muscle responds more to the availability of substrate than to the stimulus of prior exercise to synthesize its glycogen. Prolonged swimming did not affect the muscle as there was insignificant glycogen depletion. During postexercise recovery, however, it appears that soleus glycogen is depleted in favour of glycogenesis in glycogen-depleted muscles. Although lactate loading stimulated increases in direct glycogen synthesis in both rested and exercised rats, the stimulus is probably the increased plasma glucose levels as glucose loading increased direct synthesis to the same extent as in lactate-loaded rats.

Table I: Various indices of gluconeogenic glycogen synthesis in liver and muscles calculated using different methods.

Outlined is a summary of the various indices of gluconeogenic glycogen formation in liver and muscles (white gastrocnemius = White G. and red gastrocnemius = Red G.) as determined in Chapters 2-4 for rats undergoing rest, prolonged swimming and postexercise recovery with saline (rec), glucose (gluc) or lactic acid (lac) infusion. Glyconeogenic indices (in mg/g) as traced by $^{14}CO_2$ (liver only), [^{14}C-U]-lactate (^{14}C-U-Lac) or [^{14}C-1]-glucose (^{14}C-1-Gluc) were calculated as described in Chapters 2-4. *^{14}C-d.p.m.* is the difference between total recycled ^{14}C-glycogen and direct recycled ^{14}C-glycogen in [^{14}C-1]-glucose experiments. *denotes indices calculated using the average specific activities of plasma ^{14}C-glucose. *NA* denotes values which are not available as those particular protocols were not followed.

	Indices of Gluconeogenic Glycogen Synthesis (in mg/g or ^{14}C-d.p.m.)						
Method	rest + rec	rest + lac	rest + gluc	swim	swim + rec	swim + lac	swim + gluc
LIVER							
$^{14}CO_2$	0.04	0.85	NA	0.03	0.19	2.60	NA
^{14}C-U-Lac	0.02	1.26	NA	NA	0.18	2.18	NA
^{14}C-1-Gluc	0.22	0.46*	2.32	0.12	0.65	0.71*	2.24
^{14}C-d.p.m.	12137	21836	83128	4337	20869	17376	65423
SOLEUS							
^{14}C-U-Lac	-0.03	0.44	NA	NA	0.00	-0.01	NA
^{14}C-1-Gluc	0.03	0.41*	0.09	-0.27	0.20	0.35*	0.05
^{14}C-d.p.m.	2502	19060	3130	-9999	7089	9537	1300
WHITE G.							
^{14}C-U-Lac	0.03	0.68	NA	NA	0.06	1.18	NA
^{14}C-1-Gluc	0.02	0.36*	0.11	0.08	0.35	1.10*	0.50
^{14}C-d.p.m.	879	16332	3756	2882	12953	30593	14587
RED G.							
^{14}C-U-Lac	0.03	0.79	NA	NA	0.06	1.42	NA
^{14}C-1-Gluc	0.03	0.42*	0.14	0.05	0.85	1.73*	0.67
^{14}C-d.p.m.	2921	20265	4993	807	30223	46715	19698

*using average specific activity of plasma ^{14}C-glucose

Table I: Various indices of gluconeogenic glycogen synthesis in liver and muscles calculated using different methods.

	Direct Glycogen Synthesis (mg/g)						
Tissue	rest + rec	rest + lac	rest + gluc	swim	swim + rec	swim + lac	swim + gluc
LIVER	0.16	0.72	3.04	0.11	0.28	1.54	3.42
SOLEUS	1.30	3.64	3.55	1.17	1.26	3.36	2.15
WHITE G.	0.10	0.51	0.15	0.83	1.37	2.36	1.83
RED G.	0.40	1.18	1.20	1.45	2.71	4.86	3.50

Table II: Average direct liver and muscle glycogen synthesis in mg/g from all rats.

The amounts of liver and muscle (soleus, white [White G.] and red gastrocnemii [Red G.]) glycogen synthesized directly from glucose were determined in all rats undergoing rest, prolonged swimming and postexercise recovery with saline (rec), lactate (lac) or glucose (gluc) infusions. Direct synthesis was calculated as in equation 2.1 (p. 81).

WHITE AND RED GASTROCNEMII

Both white and red gastrocnemii (representative of fast-twitch glycolytic and oxidative-glycolytic muscle fibers, respectively) synthesize a portion of their glycogen via the indirect or glyconeogenic route. The source of this gluconeogenic substrate seems to be that formed locally within the muscles rather than systemic lactate. Despite the very low turnover of glycogen in the gastrocnemius muscles at rest, up to 25% (minimal estimate) of muscle glycogen was formed via the indirect route. Prolonged swimming not only resulted in significant net depletion of glycogen (50%), but also stimulated glycogenesis during and following exercise in both muscles. During a fasted postexercise recovery period, a significant proportion (20-25% based on minimal estimates) of glycogen was synthesized via glyconeogenesis despite the low lactate levels (1.3 mM at the end of exercise). Lactate loading enhanced glyconeogenic activity in the muscles in both rested and exercised animals while glucose had little stimulatory effect on either direct or indirect glycogenesis. The individual effects of exercise and lactate are additive in terms of both direct and gluconeogenic glycogen synthesis.

LIVER

Approximately two-thirds of hepatic glycogen is synthesized via the indirect or glyconeogenic route in fasted rested and exercised animals. During a fasted recovery period following exercise, liver glycogen is not repleted so as to allow for the preferential repletion of muscle glycogen. While both prior exercise and lactate

stimulate glycogenesis, the combination of the two is synergistic in the promotion of direct and indirect glycogen synthesis. Glucose appears to be a more potent stimulus of both direct and gluconeogenic hepatic glycogen synthesis than lactate.

CONCLUSION

The principal conclusion of this work is the demonstration of muscle glycogen production from glucogenic precursors (glyconeogenesis) under physiological circumstances (*in vivo*). Previous work has demonstrated the possibility or the likelihood of the occurence of this process. The studies presented here resolve previous interpretational difficulties by showing: *1)* that the substrates for glyconeogenesis appear to be formed *locally* in the tissue, *2)* the addition of lactate loads also promotes the process during recovery from exercise, as has been shown previously, and, *3)* the *local* process remains largely undectable under physiological circumstances because substrate (lactate) tracers do not equilibrate well with the tissue substrate. The use of ^{14}C-labelled glucose to generate local labelled substrate enabled observation of the intra-tissue activity.

Glyconeogenesis in the white and red gastrocnemii (fast-twitch muscles) was shown to occur during recovery from prolonged exercise of low to moderate intensity (swimming), a physiological process. It accounted for at least 25% of new glycogen synthesis during this process. The conclusions were validated by the following observations. *1)* There was no evidence of glyconeogenesis when this was assessed using a tracer ($^{14}CO_2$) which metabolically *cannot* be incorporated into muscle glycogen. (In the liver, however, this is a valid tracer.) Consistency with known metabolic facts was thus established, confirming the applicability of the assumptions on which the calculations are based. *2)* Analogous results were obtained during

lactate loading in the recovery period using *both* a systemic lactate tracer and labelled glucose. This supports the estimates based on the use of the glucose tracer in the situation (normal recovery) where the lactate tracer does not appear to be applicable.

APPENDIX A

SOURCES OF CHEMICAL REAGENTS

The anaestetic drugs used in surgery, ketamine and xylazine, were purchased from Parke-Davis and Haver, respectively. Hapalean was the source of the heparin. The sodium pentabarbital came from M.T.C. Pharmaceuticals. All of the radioactive tracer compounds, D-[^{3}H-3]-glucose, D-[^{3}H-6]-glucose, [^{14}C-1]-glucose, [^{14}C-U]-lactate and $NaH^{14}CO_3$ were purchased from New England Nuclear Research Products, and, with the exception of $NaH^{14}CO_3$, were all purified by HPLC using a Bio-Rad HPX-87P column. New England Nuclear Research Products also supplied the hyamine hydroxide and Formula 989. BDH Chemicals Ltd. supplied the following chemicals: barium hydroxide, zinc sulphate, 88% lactic acid, 90% ethanol, sodium hydroxide, potassium hydroxide, D-glucose, periodic acid, sodium bicarbonate, sodium arsenite, sodium acetate and anhydrous methanol. Dimedone, 30% sodium L-lactate, lactic acid crystals, metabolite control and the diagnostic kit for CO_2 determination were purchased from Sigma Chemical Company. Fisher Scientific Company supplied 12 N hydrochloric acid, 88% formic acid, 85% phosphoric acid, sodium benzoate, iodine, glycine and semi-carbizide hydrochloride. Universol scintillation coctail was purchased from ICN Biomedicals, Inc. Boehringer Mannheim supplied lactate dehydrogenase. Glucose SR Kit was from Medical Analysis Systems, Inc. Finally, the Dowex column resins were purchased from Bio-Rad Laboratories.

CURRICULUM VITAE

COLLEEN RYAN

#1103-751 Parkdale Avenue
Ottawa, Ontario
K1Y 1J7
Telephone: (613) 729-5143

EDUCATION

1986-1992	UNIVERSITY OF OTTAWA Candidate for Ph.D. Biochemistry (Thesis submitted)	Ottawa, Ont.
1982-1986	UNIVERSITY OF OTTAWA B.Sc. Biochemistry-Nutrition Honours *Magna Cum Laude*	Ottawa, Ont.
1979-1982	ST. PIUS X HIGH SCHOOL Grade 13 Ontario Scholar	Ottawa, Ont.

Academic Awards

1988-1991	Medical Research Council Studentship
1988-1991	University Supplementary Scholarship
1986-1988	Ontario Graduate Scholarship
1986-1988	University Supplementary Scholarship
1985	Natural Sciences and Engineering Research Council Summer Studentship
1984	Ault Scholarship
1983	Merit Scholarship
1982	Entrance Scholarship
1982	Ontario Scholar

EXPERIENCE

UNIVERSITY OF OTTAWA — Ottawa, Ont.

Student Laboratory Demonstrator

1991 Third Year Food Chemistry Laboratory

1990 Third Year Biochemistry (Metabolism)

PUBLICATIONS

Abstracts

1. Ryan, C., and J. Radziuk. Glucose metabolism during moderate exercise and recovery in rats. J. Clin. Invest. Med. 12: R-174, 1989.
(Presented to the Royal College of Physicians and Surgeons of Canada; Edmonton, Alberta, September, 1989)

2. Ryan, C., and J. Radziuk. Effect of lactate infusion on glucose and glycogen metabolism after prolonged exercise in rats. Med. Sci. Sports Exerc. 22(2): S-52, 1990.
(Presented to the American College of Sports Medicine; Salt Lake City, Utah, May, 1990)

3. Ryan, C., and J. Radziuk. Glucose, lactate and glycogen metabolism in swimming rats. J. Clin. Invest. Med. 13: B33, 1990.
(Presented to the Royal College of Physicians and Surgeons of Canada; Toronto, Ontario, September, 1990)

4. Ryan, C., and J. Radziuk. Muscle glycogenesis in swimming rats. Med. Sci. Sports Exerc. 23(4): S-98, 1991.
(Presented to the American College of Sports Medicine; Orlando, Florida, May, 1991)

5. Ryan, C., and J. Radziuk. Postexercise gluconeogenic glycogen synthesis in rat gastrocmenius. J. Clin. Invest. Med. 14(4): A31, 1991.
(Presented to the Royal College of Physicians and Surgeons of Canada; Quebec, Quebec, September, 1991)

Papers

1. Ryan, C., and J. Radziuk. Glucose dynamics and gluconeogenesis during and following prolonged swimming in rats. (Submitted to the Journal of Applied Physiology)