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THE EFFECTS OF POST-EXERCISE GLUCOSE AND ALANINE INGESTION ON PLASMA CARNITINE AND KETOSIS IN HUMANS

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SUMMARY

1. Several studies have hypothesized that alanine decreases plasma ketone body levels by increasing availability of oxaloacetate, thus allowing acetyl groups to enter the tricarboxylic acid cycle and releasing co-enzyme A (CoA).

2. Four, fasted adult males exercised at 50% of their maximal oxygen consumption for 1·5 h, then ingested 100 g of either glucose or alanine 2 h into recovery.

3. Post-exercise ketosis had developed at 2 h into recovery, as shown by a significantly elevated concentration of β-hydroxybutyrate in the plasma. At this time plasma free fatty acids were elevated above resting levels while plasma free carnitine concentrations had fallen below resting values.

4. After either alanine or glucose ingestion β-hydroxybutyrate concentrations fell to the same extent. After the alanine load free carnitine increased above that seen in the glucose trial. Following either alanine or glucose ingestion free fatty acid levels fell; they remained at resting levels in the alanine trial but decreased below rest in the glucose trial.

5. We assume that plasma carnitine concentrations largely reflect the hepatic carnitine pools; therefore, elevations in the plasma free carnitine are probably the result of an increased utilization of acetyl CoA. The significant elevation in plasma free carnitine concentration found after alanine ingestion is consistent with the hypothesis that alanine increases the oxidation of acetyl CoA by providing oxaloacetate for the tricarboxylic acid cycle.

INTRODUCTION

Acetyl co-enzyme A (CoA) represents the final common metabolite in the catabolism of all fuel sources for animals, aerobic micro-organisms and many plant tissues. While the fundamental processes that regulate the metabolism of acetyl CoA within the mitochondrion have not been elucidated, catalytic amounts of oxaloacetate are thought to be of primary importance for the oxidation of this molecule (Lehninger, 1946). Alanine is thought to inhibit ketosis during fasting and following exercise by entering the tricarboxlic acid cycle as oxaloacetate to increase the oxidation of acetyl CoA (Zammit, 1981; Koeslag, Noakes & Sloan, 1982). Carnitine buffers variations of acetyl CoA by forming acetyl carnitine (Pearson & Tubbs, 1967). Plasma esterified carnitine largely consists of the acetyl carnitine ester; therefore, a decrease in the

esterified carnitine moiety or an increase in free carnitine could reflect a decrease in the hepatic acetyl CoA pool (Parvin & Pande, 1979; Brass & Hopple, 1980; Bell & DeLucia, 1983; Bremer, 1983). We hypothesized that during post-exercise ketosis if oxaloacetate concentration limits hepatic acetyl CoA metabolism, then alanine ingestion would increase free carnitine in the plasma and reduce esterified carnitine concentrations. However, alanine ingestion may also decrease plasma free fatty acid concentrations. If this occurs the possibility exists that reducing the availability of free fatty acids for acetyl CoA formation may also result in a decrease in plasma esterified carnitine and/or an increase in the free carnitine moiety. Therefore, glucose ingestion was used as a control to show the effect of a reduction in free fatty acids without an immediate increase in mitochondrial oxaloacetate concentration.

METHODS

Four young male subjects agreed to participate in the study after undergoing informed consent procedures as established by the University of Wisconsin Medical School. All were healthy with no history of diabetes or other metabolic disorders. Subjects were studied on two occasions. Each occasion was preceded by a 48 h low-carnitine diet, followed by a 24 h period of fasting. The fast required abstention from all food intake, permitting only consumption of non-caloric beverages. The low-carnitine diet consisted of vegetables, grain products, and non-dairy liquids. All subjects reported to the laboratory early in the morning before breakfast. Each subject was studied at rest, during cycle ergometry exercise for 1·5 h and over 3·5 h of recovery. The exercise intensity was set at 50% of each subject's previously determined maximal oxygen consumption. Subjects were encouraged to drink approximately 500 ml water over each 30 min period of exercise to maintain adequate hydration and prevent haemoconcentration. 2 h into recovery 100 g of either L-alanine or glucose were ingested in a non-caffeinated and otherwise non-caloric beverage. Oxygen consumption was monitored by open-circuit spirometry using Beckman analysers (LB2 and OM11) for expired gas concentrations while a Pneumoscan respirometer was utilized for volume determinations.

Venous blood was drawn from the antecubital vein at rest, after 1·5 h of exercise and at 2·0, 2·5, 3·0 and 3·5 h into recovery. Heparin was not used because of its potential stimulatory effects on lipolysis. Approximately 10 ml blood were drawn at each time interval. A sample of 1 ml was immediately deproteinized in 5% metaphosphoric acid for β-hydroxybutyrate determination. Two 2·0 ml blood specimens were placed on ice for serum insulin and plasma glucagon determination. The glucagon sample was first treated with 0·25 ml trasylol (10000 kallikrein inactivating units/ml). The remaining blood was centrifuged at 4 °C and the supernatant was divided into 0·2 ml aliquots for the determination of plasma free fatty acids, glucose and carnitine. All plasma and serum specimens were stored at −70 °C prior to analysis. β-Hydroxybutyrate and glucose were analysed by standard enzymatic techniques (Bergemeyer, 1978). Free fatty acids were extracted from plasma and analysed by gas liquid chromatography as described by MacGee & Allen (1974). Serum insulin was measured by radioimmunoassay (Corning Medical, Medfield, MA, U.S.A.). Plasma glucagon was determined by double antibody radioimmunoassay (Cambridge Medical Diagnostics, Billerica, MA, U.S.A.). Carnitine in its free and esterified forms was analysed by the method of Parvin & Pande (1977). Total carnitine was measured as the sum of free and esterified carnitine after alkaline hydrolysis of the acyl groups in the esterified form. Free carnitine was determined on a sample prior to alkaline hydrolysis. Esterified carnitine was calculated as the difference between total and free carnitine. In addition, the protein pellet obtained from another sample was analysed for long-chain (acid-insoluble) carnitine after having undergone alkaline hydrolysis.

All values were tested relative to their change from rest by a repeated-measures analysis of variance (Winer, 1971). The resulting mean-square error was utilized to test for significant differences between the alanine and glucose conditions. In the Table and Figures a significance level of $P \leqslant 0{\cdot}05$ was selected to reject the null hypothesis. Values in the Tables and Figures are given with standard errors.

RESULTS

Table 1 contains the subject's physical characteristics and average oxygen consumption during exercise which did not differ between the alanine and glucose trials.

Plasma β-hydroxybutyrate concentrations rose significantly after 1·5 h of exercise and reached even higher values 2 h into recovery (Fig. 1*A*). Ingestion of alanine or glucose resulted in a return of β-hydroxybutyrate to pre-exercise levels within 1 h after ingestion. In the glucose trial β-hydroxybutyrate values fell significantly below

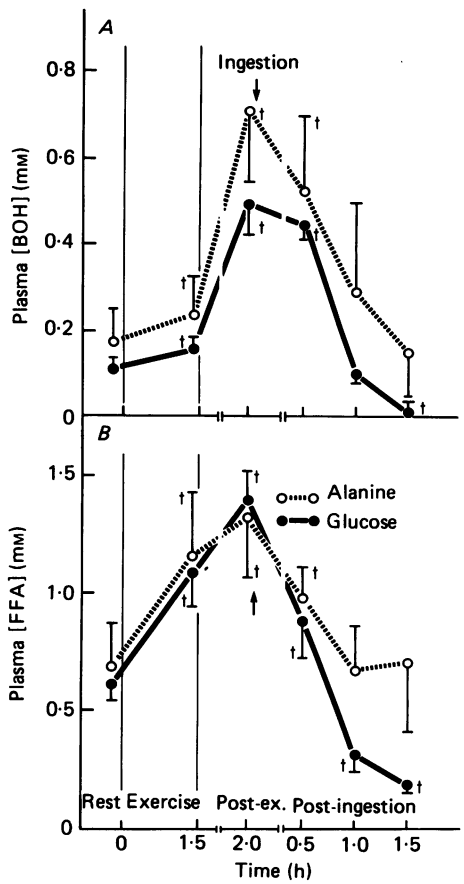

Fig. 1. Plasma β-hydroxybutyrate (*A*), and free fatty acids (FFA) (*B*) concentrations during exercise and recovery in the trials with 100 g glucose or 100 g alanine. All values are means ± S.E. of means. † Time points above or below resting values, $P \leqslant 0{\cdot}05$. $n = 4$.

TABLE 1. Physical characteristics of the subjects and their oxygen consumption during exercise

Age (years)		28·7 ± 2·6
Weight (kg)		78·7 ± 6·5
Max $\dot{V}_{O_2}$ (ml kg^{-1} min^{-1})		48·4 ± 0·4
	Glucose trial	Alanine trial
Average test $\dot{V}_{O_2}$ (% max $\dot{V}_{O_2}$)	52·5 ± 1·0	50·1 ± 1·8

All values represent means ± S.E. of means. $n = 4$.

resting levels 1·5 h post-ingestion; however, at no time did β-hydroxybutyrate values differ significantly after ingestion of either substance.

Plasma free fatty acid concentrations (Fig. 1*B*) increased significantly in both trials over the 1·5 h exercise period; they were maintained at elevated levels 2 h into recovery in both trials. Glucose ingestion resulted in free fatty acid concentrations

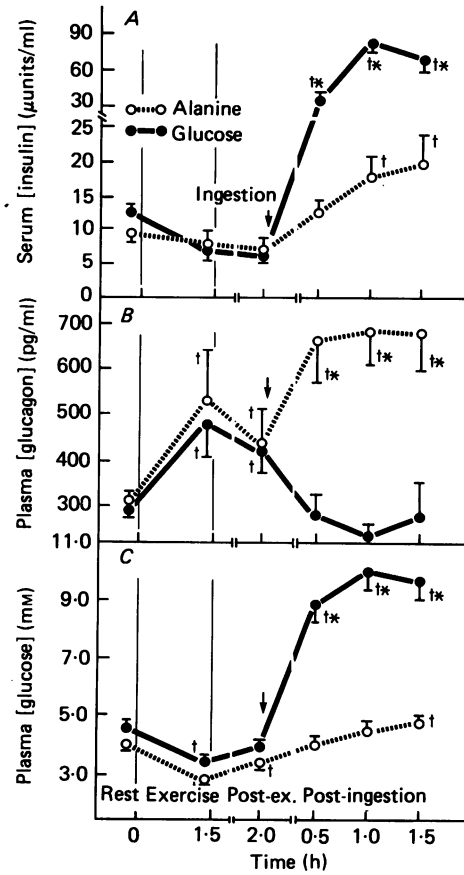

Fig. 2. Serum insulin (*A*), plasma glucagon (*B*) and glucose (*C*) concentrations during exercise and recovery in the glucose and alanine trials. All values are means ± S.E. of means. * Alanine *vs.* glucose trial, $P \leqslant 0{\cdot}05$. † Time points above or below resting values, $P \leqslant 0{\cdot}05$. $n = 4$.

falling to values significantly below rest at 1·0 and 1·5 h post-ingestion. Alanine feeding also decreased the free fatty acid concentrations significantly, but these values returned only to resting values by 1·5 h post-ingestion.

In both trials insulin concentrations showed a trend toward decreasing below resting levels immediately after exercise, falling further below resting values 2 h into recovery (Fig. 2*A*). However, these values were not statistically significantly different from rest. The ingestion of glucose resulted in a very significant increase in insulin by 0·5 h post-ingestion. Insulin levels remained elevated above those at rest during the remaining hour of measurement. Insulin rose to a lesser extent after the

alanine load and reached values which were significantly above resting levels at 1·0 and 1·5 h post-ingestion.

During the exercise and recovery period plasma glucagon concentrations in both trials were elevated significantly above pre-exercise values (Fig. 2*B*). These values remained significantly elevated above those at rest after alanine ingestion, but fell rapidly to resting levels after the glucose load.

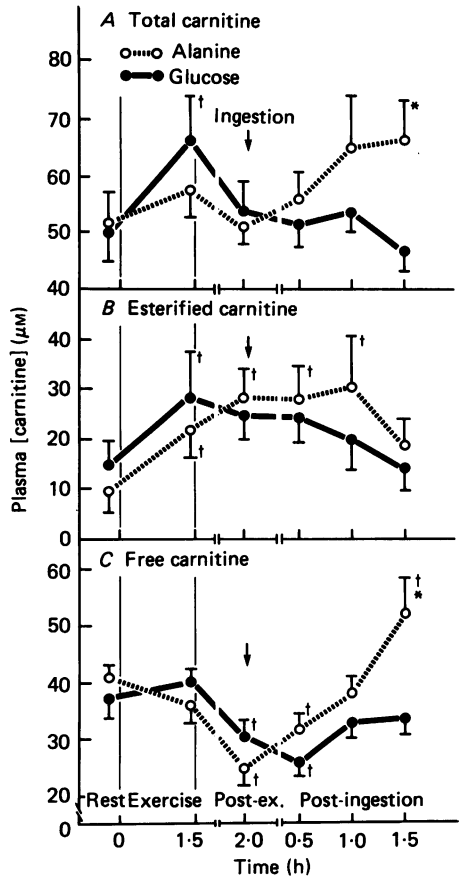

Fig. 3. Total carnitine (*A*), esterified carnitine (*B*) and free carnitine (*C*) concentrations in plasma during exercise and recovery in the glucose and alanine trials. All values are means ± S.E. of means. * Alanine *vs.* glucose trial, $P \leqslant 0{\cdot}05$. † Time points above or below resting values, $P \leqslant 0{\cdot}05$. $n = 4$.

Plasma glucose levels decreased significantly with exercise in both conditions, but returned toward rest after 2 h of recovery (Fig. 2*C*). Glucose increased significantly in response to both ingestions. The elevation in glucose in the alanine trial did not reach significance until 1·5 h post-ingestion but, as expected, after glucose ingestion values were already significantly above resting levels at 0·5 h.

Fig. 3 shows the responses of plasma carnitine and its esters to prolonged exercise and subsequent glucose or alanine ingestion. Total carnitine (Fig. 3*A*) appeared to increase with exercise in both trials; however, the increase was statistically significant

only in the glucose trial. While glucose ingestion resulted in total carnitine concentrations which were maintained at resting levels, the alanine load resulted in significant elevations in total carnitine above those at rest which reached values significantly different from those in the glucose trial.

Plasma esterified carnitine (Fig. 3*B*) was elevated significantly above the resting

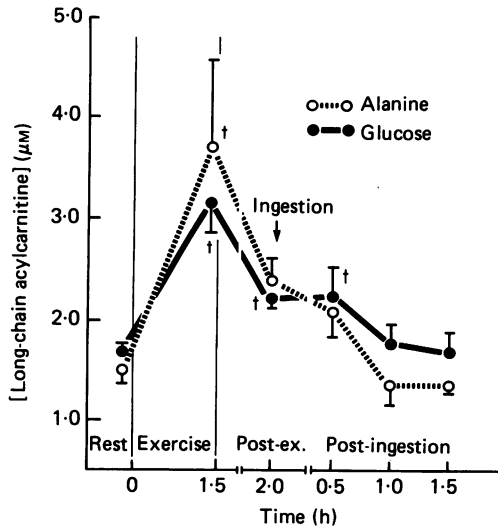

Fig. 4. Plasma long-chain acylcarnitine concentrations during exercise and recovery in the glucose and alanine trials. All values are means ± S.E. of means. † Time points above or below resting values, $P \leqslant 0{\cdot}05$. $n = 4$.

level after 1·5 h of exercise in both trials and continued to increase after the alanine load to 1 h post-ingestion, after which it returned to resting levels. After glucose ingestion esterified carnitine returned immediately toward resting values.

Free carnitine was maintained at resting values during exercise (Fig. 3*C*), but fell significantly below those at rest 2 h into recovery in both trials. Free carnitine returned to resting levels after glucose ingestion. Alanine ingestion resulted in significant elevations of free carnitine above resting levels. This increase was significantly greater than that after the glucose load.

Plasma long-chain acylcarnitine increased significantly above the resting level after 1·5 h of exercise in both groups (Fig. 4). Post-exercise, these values fell towards resting levels 2 h into recovery and continued to decline to resting levels after ingestion of either substance.

DISCUSSION

This study utilizes variations in the plasma carnitine pool to demonstrate the hypothesized importance of oxaloacetate in the regulation of acetyl CoA and therefore β-hydroxybutyrate production. Specifically, alanine, an oxaloacetate precursor, increased plasma free carnitine concentration above the level of that after glucose ingestion, while decreasing β-hydroxybutyrate to the same extent as in the glucose trial (Fig. 3*A* and *C*). These changes in β-hydroxybutyrate and carnitine occurred

with plasma free fatty acid concentrations decreasing below resting values after the glucose load, while remaining at resting levels after alanine ingestion (Fig. 1*B*). These findings would support the conclusions of other authors who have suggested that alanine decreases plasma ketone body levels post-exercise (Koeslag *et al.* 1982) or after somatostatin infusion (Nosadini, Alberti, Johnson, Del Prato, Marescotti & Dune, 1981) by increasing oxaloacetate availability for citrate synthase-catalysed condensation with acetyl CoA.

Glucose ingestion resulted in no significant change in either free or esterified carnitine (Fig. 3*B* and *C*). However, esterified carnitine did appear to be falling below pre-exercise values after glucose ingestion. This decrease in esterified carnitine could be due to a drop in free fatty acid concentration. The lack of a change in free carnitine with an apparent fall in esterified carnitine would suggest that the hepatic oxaloacetate concentration is not influenced by glucose feeding during the recovery period, and that decreases in mitochondrial acetyl CoA levels are mainly the result of a fall in free fatty acids. Glucose would not be expected to have affected the liver supply of oxaloacetate since much of an oral glucose load would go toward muscle glycogen resynthesis during the recovery period (Maehlum, Felig & Wahren, 1978).

It is also important to discuss variations in the plasma carnitine pool prior to the ingestion period. When exercise stops, the release of alanine, pyruvate and lactate from exercising muscle rapidly returns to resting levels, while hepatic gluconeogenesis remains well above resting levels (Wahren, Felig, Handler & Alborg, 1973; Zinman, Murray, Vranic, Albisser, Leible, McClean & Marliss, 1977; Vranic & Kawamori, 1979). This would decrease hepatic concentrations of 3-carbon units available for oxaloacetate formation within the tricarboxylic acid cycle. The large decrement in plasma free carnitine in this study at 2 h post-exercise might reflect a movement of free carnitine out of the plasma compartment in order to buffer elevations in hepatic acetyl CoA brought about by declining oxaloacetate concentrations (Fig. 3*C*). The redistribution of plasma free carnitine to the liver would also be an important mechanism in protecting the hepatic free CoA pool during a period when the oxidation of the acetyl moiety is limited.

It is possible that variations in plasma carnitine could be the result of changes in carnitine synthesis. However, we do not feel this is the case. First, as mentioned in the Introduction, esterified carnitine is largely composed of acetyl carnitine (Valkner & Bieber, 1982) and any reaction resulting in changes in plasma carnitine probably reflects a change in the acetyl carnitine pool (Parvin & Pande, 1979; Brass & Hopple, 1980; Bremer, 1983). Secondly, β-hydroxybutyrate formation is largely the result of incomplete acetyl CoA metabolism. Plasma elevations in this ketone body are closely related to that of esterified carnitine during fasting; this strongly suggests that the plasma carnitine pool is in equilibrium with acetyl CoA through the formation of acetyl carnitine (Parvin & Pande, 1979; Brass & Hopple, 1980; Bell & Delucia, 1983).

Although it is well accepted that the rate of ketone body production is determined by the level of acetyl CoA within the mitochondrion, controversy exists concerning the regulatory role of carnitine acyltransferase in this process. This enzyme is purported to control the entry of free fatty acids into the mitochondrion for ketone body

formation (McGarry & Foster, 1979). The regulatory role of this enzyme, as reflected by alterations in plasma long-chain acylcarnitine, cannot be determined in this study (Fig. 4). The observed decrease in β-hydroxybutyrate after either ingestion (Fig. 1*A*) could be due to an inactivation of the acyltransferase. However, the fall in this ketone body may be merely the result of a reduction in free fatty acids following glucose ingestion and an increased oxaloacetate supply following alanine ingestion, without a change in transferase activity. This study provides direct evidence to support the concept that it may be 'intrahepatic concentrations or flux of substrates that provides the over-riding control of ketone body synthesis' (Koeslag *et al.* 1982). Specifically, the above changes in plasma carnitine reflect the physiological role that intrahepatic concentrations or flux of substrates may have in regulating the hepatic acetyl CoA pool.

In conclusion, alterations in plasma carnitine to post-exercise alanine and glucose ingestion are consistent with the hypothesized role of oxaloacetate in regulating hepatic acetyl CoA and β-hydroxybutyrate production.

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REFERENCES

BELL, F. P. & DELUCIA, A. J. (1983). Plasma and liver carnitine (free and esterified) levels and their interrelationships in moderately hypercholesterolemic monkeys (*Macaca arctoides*). *Canadian Journal of Biochemistry and Cell Biology* **61**, 328–332.

BERGEMEYER, H. U. (1965). *Methods in Enzymatic Analysis*, pp. 59–62 and 260–262. New York: Academic Press.

BRASS, E. P. & HOPPLE, C. L. (1980). Relationship between acid-soluble carnitine and coenzyme A pools *in vivo*. *Biochemical Journal* **190**, 495–504.

BREMER, J. (1983). Carnitine metabolism and functions. *Physiological Reviews* **63**, 1420–1480.

BROOKS, D. E. & MCINTOSH, J. E. (1975). Turnover of carnitine by rat tissues. *Biochemical Journal* **148**, 439–445.

KOESLAG, J. H., NOAKES, T. D. & SLOAN, A. W. (1982). The effects of alanine, glucose and starch ingestion on the ketosis produced by exercise and by starvation. *Journal of Physiology* **325**, 363–376.

LEHNINGER, A. L. (1946). A qualitative study of the products of fatty acid oxidation in liver suspensions. *Journal of Biological Chemistry* **164**, 291–306.

MACGEE, J. & ALLEN, K. G. (1974). Preparation of methyl esters from the saponifiable fatty acids in small biological specimens for gas-liquid chomatographic analysis. *Journal of Chromatography* **100**, 35–42.

MCGARRY, J. D. & FOSTER, D. W. (1979). In support of the role of malonyl-CoA and carnitine acyltransferase I in the regulation of hepatic fatty acid oxidation and ketogenesis. *Journal of Biological Chemistry* **254**, 8163–8168.

MAEHLUM, S., FELIG, P. & WAHREN, J. (1978). Splanchnic glucose and muscle glycogen metabolism after glucose feeding during post-exercise recovery. *American Journal of Physiology* **235**, E255–260.

NOSADINI, R., ALBERTI, K. G. M. M., JOHNSON, D. G., DEL PRATO, S., MARESCOTTI, C. & DUNE, E. (1981). The antiketogenic effect of alanine in normal man: evidence for an alanine–ketone body cycle. *Metabolism* **30**, 563–332.

PARVIN, R. & PANDE, S. V. (1977). Microdetermination of (−)-carnitine and carnitine acetyltransferase activity. *Analytical Biochemistry* **79**, 190–201.

PARVIN, R. & PANDE, S. V. (1979). Enhancement of mitochondrial carnitine and carnitine acylcarnitine translocase-mediated transport of fatty acids into liver mitochondria under ketogenic conditions. *Journal of Biological Chemistry* **254**, 5423–5429.

PEARSON, D. J. & TUBBS, P. K. (1967). Carnitine and its derivatives in rat tissues. *Biochemical Journal* **105**, 953–963.

VALKNER, K. J. & BIEBER, L. L. (1982). Short-chain acylcarnitines in human-blood and urine. *Biochemical Medicine* **28**, 197–203.

VRANIC, M. & KAWAMORI, R. (1979). Essential roles of insulin and glucagon in regulating glucose fluxes during exercise in the dog. Mechanism of hypoglycemia. *Diabetes* **28**, suppl. 1, 45–52.

WAHREN, J., FELIG, P., HENDLER, R. & AHLBORG, G. (1973) Glucose and amino acid metabolism during recovery after exercise. *Journal of Applied Physiology* **34**, 838–845.

WINER, B. J. (1971). *Statistical Principles in Experimental Design*, 2nd. edn., pp. 514–539, New York: McGraw Hill.

ZAMMIT, V. A. (1981). Intrahepatic regulation of ketogenesis. *Trends in Biochemical Science* **6**, 46–49.

ZINMAN, B., MURRAY, F. T., VRANIC, M., ALBISSER, A. M., LEIBLE, B. S., MCCLEAN, P. A. & MARLISS, E. B. (1977). Glucoregulation during moderate exercise in insulin treated diabetics. *Journal of Clinical Endocrinology and Metabolism* **45**, 641–652.