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Metabolic Response to Exercise

# PLASMA FREE AND ESTERIFIED CARNITINE LEVELS IN THE STREPTOZOTOCIN DIABETIC RAT AFTER A SINGLE BOUT OF EXERCISE

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## ABSTRACT

**Broderick TL, Nadeau A**. Plasma free and esterified carnitine levels in the streptozotocin rat after a single bout of exercise. ***JEP****online* 2006;9(3):17-23. Clinical diabetes is associated with disturbances in carnitine metabolism during exercise. In type 1 diabetic patients, the typical reduction in plasma free carnitine (FC) is absent during an acute session of exercise. To establish an experimental model of study, male Wistar rats were divided into a control (n=8) and a diabetic (n=8) group. Diabetes was induced with streptozotocin (STZ) at the dose of 50 mg/kg body weight. One week following the induction of diabetes, exercise was performed on a treadmill at 22 m/min for 60 min at an incline of 8°. Arterial blood was collected at rest and immediately following exercise in previously cannulated rats. In control rats, compared to rest, FC levels decreased significantly after 60 min of exercise. In diabetic rats, on the other hand, the decrease in FC levels was not observed after exercise. Plasma esterified carnitine levels increased in both groups post-exercise, but this increase was non significant from resting levels. Our results indicate that the plasma FC response at the end of acute exercise in the STZ-model of diabetes is consistent with the observations in type 1 diabetic patients. This model may thus be suited to examine the disturbances in carnitine metabolism during exercise typically reported in diabetic patients.

**Key Words**: Exercise, Metabolism, Diabetes, Insulin, Glucose

## INTRODUCTION

Carnitine (β-hydroxy-γ-trimethylaminobutyric acid) is an essential cofactor in the transfer and oxidation of fatty acids in the mitochondria of highly oxidative tissues (1). The role of carnitine in fatty acid metabolism becomes increasingly important during exercise, as fatty acids are a major source of energy for contracting muscle (2). As the demand for fatty acids increases, the requirement for carnitine in working muscle also increases and its use is reflected by a reduction in free carnitine (FC) in the plasma. Also reflecting this state is a heightened acylcarnitine production from fatty acid esterification with subsequent appearance of esterified carnitine (EC) in the plasma (2-4). The association between carnitine and fatty acid metabolism is supported by the observation that hyperinsulinemia in exercising humans, achieved by intravenous infusion, suppresses fatty acid metabolism and attenuates the changes in both FC and EC (5).

The effects of exercise on plasma carnitine metabolism have also been reported in patients with diabetes mellitus (6,7). Since this condition is associated with alterations in whole body substrate utilization (8), disturbances in carnitine metabolism are particularly evident. In type 1 diabetic patients, we have shown that the typical reduction in plasma FC was not observed (6), whereas in type 2 diabetic patients, the increase in EC was attenuated during exercise (7). Although these changes remain unexplained, a greater reliance on glucose as energy substrate and defects in fatty acid metabolism, frequent in type 1 and type 2, respectively, may account for these changes (6,8,9). Part of the difficulty in explaining these disturbances in carnitine metabolism during exercise also stems from the lack of an appropriate experimental model to investigate the impact of diabetes.

Indeed, studies intended to investigate from a mechanistic approach the changes in carnitine metabolism that occur in diabetes must consider an experimental model. The streptozotocin (STZ)-induced diabetic rat is undoubtedly the most widely used model and has provided valuable information on the diabetic state (10). An attractive feature of this model is that the severity of diabetes can be tailored by adjusting the dose of STZ and if necessary implementing a diet rich in fat to produce a state of insulin resistance (11). By manipulating these variables in rodents, it is therefore possible to induce either type 1 or type 2-diabetes. In the present study, we chose to induce an insulinopenic state in rats that closely resembles type 1 diabetes and determined whether a plasma carnitine profile can be obtained during acute exercise, and whether this response closely resembles that previously observed in the type 1 diabetic patient (6).

## METHODS

### Animals And Induction Of Diabetes

The animals used in this study were cared for according to the recommendations in the Canadian Council on Animal Care's "Guide to the Care and Use of Experimental Animals". Male Wistar rats (n=16) weighing 195 g were used for this study. Each animal was individually housed at 23 °C under standard lighting (05.00-19.00 hours) and allowed free access to rat chow and tap water. Diabetes was induced in ether-anesthetized rats (n=8) by a single penile vein injection of STZ at the dose of 50 mg/kg dissolved in citrate buffer, pH 4.5. STZ is selectively toxic to the insulin-producing β-cells of the pancreatic islets. Destruction of the cells that regulate blood glucose levels results in hyperglycemia, the hallmark of diabetes. Control rats (n=8) received an equivalent amount of citrate buffer.

One week later, after an overnight fast, diabetes was confirmed by the level of glucose assessed in tail-blood with Dextrostix strips read with a Glucometer (Ames Division, Miles Laboratories, Rexdale,

Ontario, Canada). With the concentration of STZ used, all rats developed severe diabetes with plasma glucose levels ranging between 19 and 28 mmol/L.

**Exercise Protocol**

The animals were first familiarized to the treadmill for 1 week by exposure to daily 10 min runs. Thereafter, rats were anaesthetized and the left carotid artery was cannulated (12) to later obtain blood samples in unrestrained animals. Two- to three days later, after an overnight fast, exercise was performed on motor-driven treadmill (Quinton Instruments, model 42-15, Seattle, Washington, U.S.A.) by both control and diabetic animals. The duration of the exercise bout was 60 min and the treadmill speed was set at 22 m/min with a grade set at 8°, corresponding to an estimated oxygen cost of ~ 58 ml/kg/min (13). Arterial blood samples were obtained at rest and immediately after exercise for later measurement of plasma glucose, insulin, and carnitine. The blood was quickly centrifuged after the exercise sessions at 3,000 rpm and the plasma separated and frozen at -80ºC until analysis.

**Assay Methods**

Plasma glucose was determined spectrophotometrically by an enzymatic method using hexokinase and glucose-6-phosphate dehydrogenase (14). Plasma insulin was determined by radioimmunoassay with rat insulin as standard and polyethylene glycol separation (15). Plasma carnitine was measured by the radioenzymatic assay described by McGarry and Foster (16) using carnitine acetyltransferase and [$^{14}$C] acetyl CoA. All assays were performed at the end of the study.

**Statistical Analyses**

Statistical analysis was performed using unpaired t-tests for comparisons between group means. Comparison of plasma values before and after exercise in each group was determined using paired t-tests. All values are reported as mean ± SD.

## RESULTS

Figure 1A shows plasma glucose levels at rest and following 60 min of exercise in control and diabetic rats. Confirming the diabetic state, glucose levels were significantly increased in diabetic rats compared to control rats. Sixty min of exercise was associated with a significant decrease in glucose levels in both groups of rats. However, at the end of exercise, glucose levels remained significantly higher in diabetic rats.

As expected, following STZ treatment, pancreatic function was altered in diabetic rats. Indeed, as illustrated in Figure 1B, plasma insulin levels before exercise were significantly lower in diabetic rats compared to control rats. After exercise, plasma insulin levels were decreased and remained significantly lower in control rats compared to diabetic rats. In diabetic rats, however, the decrease in insulin levels was not observed.

The effect of acute exercise on plasma FC levels is depicted in figure 2A. FC levels were significantly higher in control rats compared to diabetic rats. Exercise was associated with a significant decrease in FC in the control rats, whereas in diabetic rats, FC levels remained unchanged from resting values.

As shown in Figure 2B, the levels of plasma EC at rest were similar in control rats and diabetic rats. After 60 min of exercise, plasma EC levels were significantly higher in control rats compared to diabetic rats.

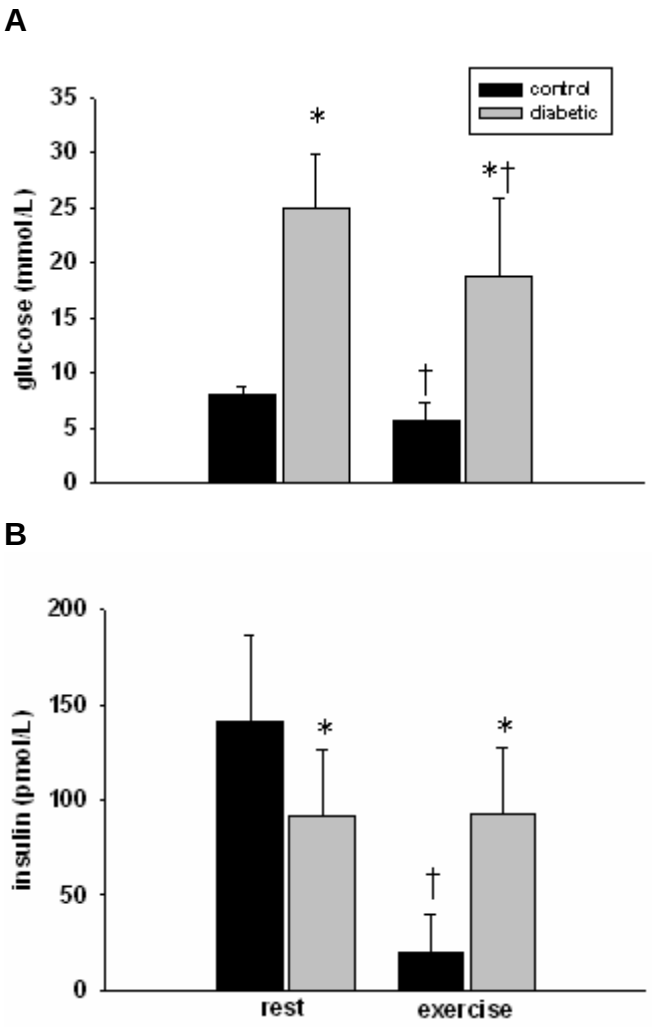


**Figure 1. Values are shown as mean ± SD for 8 rats in each group for A) plasma glucose and B) plasma insulin. * P<0.05, compared to control rats; †, P<0.05, compared to rest in respective group**

## DISCUSSION

The present study is the first to make use of a chemically-induced form of diabetes in the rat to measure the changes in plasma carnitine during acute exercise. Using the STZ-model of diabetes, measurements of plasma FC and EC were made before and immediately after 60 min of exercise. Our results demonstrate that close similarities exist in the plasma FC profile to exercise between the STZ-rat and type 1 diabetic patient. The level of plasma FC levels was decreased after exercise in the control rat, whereas they remained unchanged in the STZ-rat, a pattern that clearly mimics the response observed in both the non-diabetic and type 1 diabetic patient, respectively. Based on these observations, the STZ-model of diabetes may be pertinent to investigate the metabolic disturbances in carnitine metabolism during exercise seen in human type 1 diabetes. That this model is suited to study carnitine metabolism is further supported by the findings that the changes in both plasma glucose and insulin in the STZ-model are similar to the responses reported in the type 1 diabetic patient (6).

Since we have succeeded in developing a model for the study of plasma carnitine metabolism in an exercise setting using the diabetic rat, at this point, we can only speculate on the similarity in the FC response between the STZ-rat and type 1 diabetic patient. Of interest, though care must be taken in this interpretation, is the role of insulin on fatty acid metabolism. The suppressive role of insulin on adipose tissue lipolysis has been suggested to explain the FC response in type 1 diabetic patients (5,6). In diabetic patients receiving exogenous insulin, absorption from injection sites results in over-insulinization and inhibition of lipolysis (17,18). This limits the availability of free fatty acids resulting in a preferential use of glucose as energy source (19-21). An elevated respiratory exchange ratio observed in hyperinsulinemic type 1 diabetic patients is consistent with the preferential utilization of carbohydrates as an energy substrate during exercise (6). A hyperinsulinemic state induced by an intravenous infusion of insulin in the non-diabetic patient is also associated with a limited blood level of free fatty acids and reduced FC response during exercise (5).

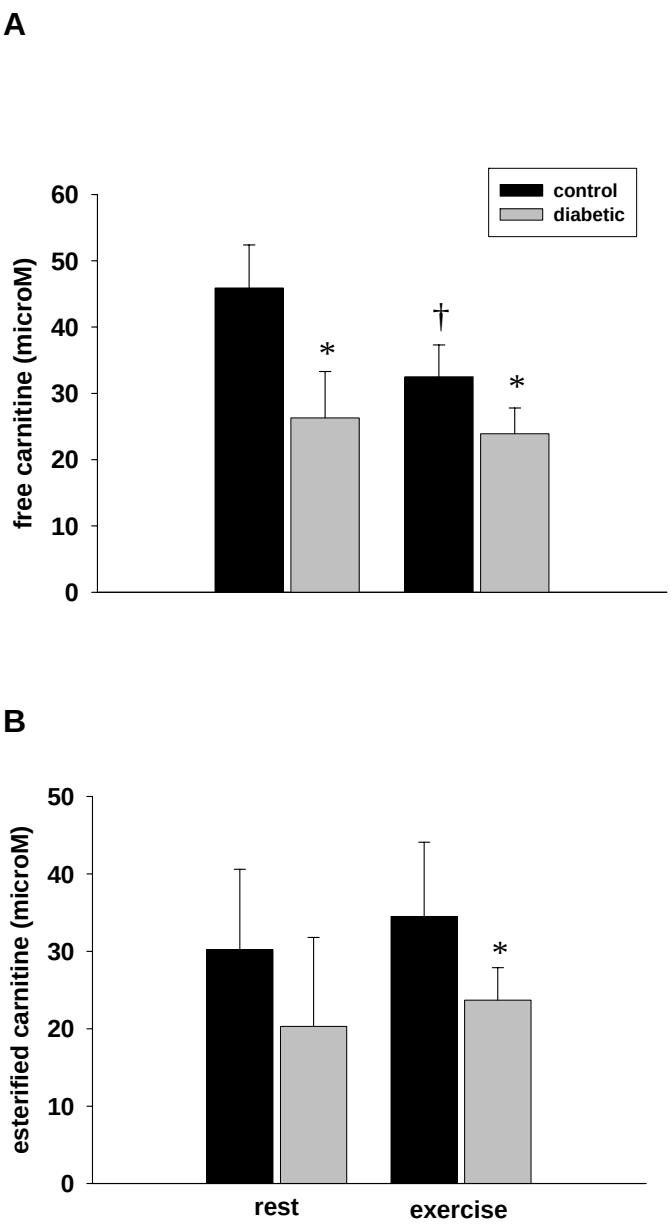


**Figure 2. Values are expressed as mean ± SD for 8 rats in each group. * P<0.05, compared to control rats; †, P<0.05, compared to rest in respective group**

In the present study, however, diabetic rats were not hyperinsulinemic. Pre-exercise insulin levels were lower in diabetic rats compared to control rats and remained unchanged after exercise, a profile that would inevitably be associated with an elevated blood level of free fatty acids (22). Under these conditions, the FC response in diabetic rats is clearly not consistent with the role of insulin in suppressing free fatty acid mobilization. An intriguing question then arises in what explains the altered FC response in the diabetic rat? One possibility is that the hyperglycemic state of diabetic animals is associated with a preferential use of glucose. Indeed, glucose uptake and oxidation by exercising muscle are stimulated under conditions of hyperglycemia (20,21).

While more studies are required to delineate the effects of STZ on carnitine metabolism during exercise, it should be clear that the mechanisms explaining the FC response based solely substrate oxidation and plasma changes are assumptions. This is because 99 % of total carnitine is located in the intracellular pool and the relation between carnitine and its function in fatty acid flux is highly sensitive to the mitochondrial metabolic state (1). During exercise, the rate of fatty acid oxidation in muscle is related to the concentration of fatty acids into the cytoplasm to which the mitochondria is exposed rather than the concentration of free fatty acids in the plasma. As the availability of fatty acids in the plasma is reduced with hyperinsulinemia, it remains unknown whether this would limit the involvement of carnitine in fatty acid oxidation. Clearly, future studies are warranted to elucidate this possibility.

## CONCLUSIONS

In conclusion, using the STZ-model of diabetes, we were able to demonstrate that acute exercise presents a plasma carnitine pattern that is similar to that reported in the type 1 diabetic patient. The STZ-diabetic rat model seems appropriate for studies requiring extensive tissue sampling not possible in humans to elucidate the disturbances in carnitine metabolism.

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