Noninvasive Assessment of Exercise-Related Intramyocellular Acetylcarnitine in Euglycemia and Hyperglycemia in Patients With Type 1 Diabetes Using $^1$H Magnetic Resonance Spectroscopy

A randomized single-blind crossover study

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OBJECTIVE — Intramyocellular acetylcarnitine (IMAC) is involved in exercise-related fuel metabolism. It is not known whether levels of systemic glucose influence IMAC levels in type 1 diabetes.

RESEARCH DESIGN AND METHODS — Seven male individuals with type 1 diabetes performed 120 min of aerobic exercise at 55–60% of $\dot{V}O_2max$ randomly on two occasions (glucose clamped at 5 or 11 mmol/l, identical insulinemia). Before and after exercise, IMAC was measured by $^1$H magnetic resonance spectroscopy in muscle vastus intermedius.

RESULTS — Postexercise levels of IMAC were significantly higher than pre-exercise values in euglycemia (4.30 ± 0.20 mmol/l, $P < 0.001$) and in hyperglycemia (2.44 ± 0.33 mmol/l, $P = 0.01$) and differed significantly according to glycemia ($P < 0.01$). The increase in exercise-related levels of IMAC was significantly higher in euglycemia (3.97 ± 0.45 mmol/l) than in hyperglycemia (1.71 ± 0.50 mmol/l, $P < 0.01$).

CONCLUSIONS — The increase in IMAC associated with moderate aerobic exercise in individuals with type 1 diabetes was significantly higher in euglycemia than in hyperglycemia.

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Intramyocellular acetylcarnitine (IMAC) is involved in the regulation of fat and carbohydrate oxidation in skeletal muscle during moderate aerobic exercise (1). Although carnitine metabolism appears comparable in patients with type 1 diabetes and healthy control subjects (2), it is not known whether IMAC is related to variations in fuel metabolism observed during exercise under differing glycemic levels in type 1 diabetes (3). Given the controversial results from previous studies linking IMAC to increased $\beta$-oxidation (4) but also to high glycolytic flux (1), the aim of the present study was to assess exercise-related concentrations of IMAC noninvasively by $^1$H magnetic resonance spectroscopy ($^1$H MRS) (5,6) in patients with type 1 diabetes in euglycemia and hyperglycemia.

RESEARCH DESIGN AND METHODS — This was a randomized single-blind crossover study (3). Seven physically active individuals with type 1 diabetes without relevant diabetes-associated complications were investigated (mean ± SEM age 33.5 ± 2.4 years, diabetes duration 20.1 ± 3.6 years, A1C 6.7 ± 0.2%, BMI 24.3 ± 0.4 kg/m², and $\dot{V}O_2max$ 50.3 ± 4.5 ml·min⁻¹·kg⁻¹). All were treated with continuous subcutaneous insulin infusion (insulin dose 0.61 ± 0.03 units·kg⁻¹·day⁻¹). Participants performed 120 min of exercise at 55–60% of $\dot{V}O_2max$ on two occasions in random order with glucose clamped at 5.4 ± 0.5 mmol/l (euglycemia) or 11.0 ± 0.3 mmol/l (hyperglycemia). Insulin infusion (7 mU·m⁻²·min⁻¹, range 5–8.2) was equal during both conditions (3). Muscle spectra were obtained with short echo time $^1$H MRS from muscle vastus intermedius 81 ± 2 and 83 ± 2 mm after exercise in euglycemia and hyperglycemia, respectively (NS). Acquisition parameters were clinical 1.5-T scanner (GE Medical Systems, Waukesha WI), localized volume of 11 × 12 × 18 mm³, point-resolved spectroscopy localization, repetition time 3 s, echo time 20 ms, two spectra of 128 acquisitions, and water presaturation. Spectra were analyzed using TDFDFit (7) with a fit strategy optimized for the acetylcarnitine-peak. Acetylcarnitine content is expressed in absolute arbitrary units (a.u.) relative to the water signal intensity. Parameters are expressed as means ± SEM (median and range where appropriate).

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RESULTS — Figure 1A displays the summed spectra before and after exercise and corresponding differences with the acetyl peak of IMAC at 2.13 ppm. Pre-exercise levels of IMAC did not differ significantly according to the glycemic levels (0.33 ± 0.21 and 0.73 ± 0.33 a.u. for euglycemia and hyperglycemia, respectively; NS). Postexercise levels of IMAC were significantly higher than pre-exercise values in euglycemia (4.30 ± 0.54 a.u.; \( P < 0.001 \)) and in hyperglycemia (2.44 ± 0.53 a.u.; \( P = 0.01 \)). In addition, postexercise levels differed significantly according to glycemia \( (P < 0.01) \). As shown in Fig. 1B, this translated into a significantly higher increase of exercise-related levels of IMAC in euglycemia (3.97 ± 0.45 a.u.) compared with that in hyperglycemia (1.71 ± 0.50 a.u.; \( P < 0.01 \)).

CONCLUSIONS — In the present study, levels of IMAC were assessed non-invasively in skeletal muscle of individuals with type 1 diabetes in euglycemia and hyperglycemia before and after moderate aerobic exercise. The principal finding was a significantly higher increase in IMAC in euglycemia than in hyperglycemia.

The effect of euglycemia and hyperglycemia on exercise-related substrate oxidation has also been assessed in healthy individuals (8), however, without determination of IMAC. Still, previous reports do not suggest genuinely different levels of acylcarnitine in patients with type 1 diabetes compared with nondiabetic control subjects (2). Conversely, a recent study in patients with type 1 diabetes investigated levels of IMAC during exercise in euglycemia but with differing insulin levels (9). The authors reported a similar increase in IMAC in euglycemia and hyperinsulinemia, implying that the flux through the pyruvate dehydrogenase complex (PDC) is independent of insulin concentrations (9). Taken together, these results indicate that in individuals with type 1 diabetes, levels of IMAC are influenced by the availability of energy substrates, in particular systemic glucose, whereas levels of insulin seem to have less influence. Of note, IMAC has been shown to increase after a few minutes of high-intensity exercise (10). This result has been hypothesized to be due to the production of acetyl-CoA exceeding its utili-
Glycemia and acetyl carnitine in exercise

zation by the Krebs cycle, the acetyl groups consequently being transferred to carnitine by carnitine acetyltransferase, which results in increases in IMAC as well as in free CoA (1). Although IMAC may thus reflect the imbalance of the fluxes through the PDC and the Krebs cycle in high-intensity exercise, its role during exercise at lower intensity or at rest is less clear. Interestingly, previous studies in healthy individuals (4) have suggested an association of IMAC with increased exercise. The present study was performed in male individuals with type 1 diabetes exclusively, and the complex study design along with the limited resources did not allow us to include a control group of healthy individuals. Statements referring to a general population thus clearly remain restrictive.

In summary, in individuals with well-controlled type 1 diabetes, an exercise-related increase in IMAC was detected in both glycemic conditions, but the effect was significantly higher in euglycemia than in hyperglycemia. These findings point to a role of IMAC as a marker of exercise-related fuel metabolism in skeletal muscle.

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References
1. Stephens FB, Constantin-Teodosiu D, Greenhaff PL. New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. J Physiol 2007; 581:431–444
2. Mamoulakis D, Galanakis E, Dionysopoulou E, Evangelou A, Sbrakas S. Carnitine deficiency in children and adolescents with type 1 diabetes. J Diabetes Complications 2004; 18:271–274
3. Jenni S, Oetliker C, Allemann S, Ith M, Tappy L, Wuerth S, Egger A, Boesch C, Schnetter P, Diem P, Christ E, Stettler C. Fuel metabolism during exercise in euglycemia and hyperglycaemia in patients with type 1 diabetes mellitus—a prospective single-blinded randomised crossover trial. Diabetologica 2008; 51:1457–1465
4. Tsintzas K, Chokkalingam K, Jewell K, Norton L, Macdonald IA, Constantin-Teodosiu D. Elevated free fatty acids attenuate the insulin-induced suppression of PDK4 gene expression in human skeletal muscle: potential role of intramuscular long-chain acyl-coenzyme A. J Clin Endocrinol Metab 2007; 92:3967–3972
5. Kreis R, Jung B, Rotman S, Slotboom J, Boesch C. Non-invasive observation of acetyl-group buffering by 1H-MR spectroscopy in exercising human muscle. NMR Biomed 1999; 12:471–476
6. White LJ, Robers AG, Vibhuti WR, J, Gasparovic CM, Petropoulos H, Brooks WM. Accumulation of acetyl groups following cycling: a 1H-MR spectroscopy study. Int J Sports Med 2006; 27:100–104
7. Slotboom J, Boesch C, Kreis R. Versatile frequency domain fitting using time domain models and prior knowledge. Magn Reson Med 1998; 39:899–911
8. Coyle EF, Hamilton MT, Alonso JG, Montain SJ, Ivy JL. Carbohydrate metabolism during intense exercise when hyperglycemic. J Appl Physiol 1991; 70:834–840
9. Chokkalingam K, Tsintzas K, Norton L, Jewell K, Macdonald IA, Mansell P. Exercise under hyperinsulinaemic conditions increases whole-body glucose disposal without affecting muscle glycogen utilisation in type 1 diabetes. Diabetologia 2007; 50:414–421
10. Harris RC, Foster CV, Hultman E. Acetyl-carnitine formation during intense muscular contraction in humans. J Appl Physiol 1987; 63:440–442