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Short Communication

How swimming affects plasma insulin and glucose concentration in thoroughbreds: A pilot study

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Highlights

- Training increased insulin-stimulated glucose uptake in skeletal muscle, with a decrease in plasma glucose concentration.
- Plasma levels of insulin and glucose were evaluated in 12 horses during an IV glucose tolerance test (IVGTT).
- The IVGTT was performed before and 1 month after training in a swimming pool.
- Compared to pre-training values, post-training area under the curve increased for plasma glucose, but not for plasma insulin.
- Low-intensity prolonged swimming exercise might improve glucose metabolism in horses.

Abstract

Low intensity exercise increases insulin-stimulated glucose uptake in skeletal muscle and decreases its plasma concentration. In this study, plasma insulin and glucose concentrations were evaluated 5 min before and 5, 15, 25, 35, 45 and 60 min after an IV bolus of glucose in 12 Thoroughbreds before and after 1 month of submaximal aquatraining exercise, monitored using heart rate and blood lactate. Plasma glucose concentrations were evaluated using a colorimetric enzymatic method, and plasma insulin concentrations with a solid-phase radioimmunoassay method. Pre-training plasma glucose concentrations at 15, 25 and 35 min, area under the glucose curve and peak glucose concentration were significantly higher than post-training values ($P<0.05$). Baseline pre-training plasma insulin concentrations were significantly lower than in the post-training period, and plasma insulin was significantly higher at 45 and 60 min in the pre-training

period than the post-training period. These results indicate that aquatraining could improve insulin-glucose metabolism in horses.

Keywords: Equine; Exercise; Plasma glucose; Plasma insulin; Swimming

Swimming pools are becoming popular for equine rehabilitation because aquatic exercise is believed to be beneficial for muscle activation and joint mobility. Low intensity training increases insulin-stimulated glucose uptake in skeletal muscles; uptake rate is limited by glucose transport across the sarcolemma (Richter and Hargreaves, 2013). This mechanism is almost exclusively mediated by insulin-stimulated glucose protein transporters (Duehlmeier et al., 2010). In humans, the concentration of these transporters increases with exercise and is associated with a concomitant increase in insulin-stimulated glucose transport (Richter and Hargreaves, 2013). In horses, this is accompanied by decreased plasma glucose and insulin after a period of training (McCutcheon et al., 2002).

The aim of this study was to determine the effect of submaximal aquatraining exercise on plasma glucose and insulin values. The study was regulated by Italian Animal Care (DL 116/92) and approved by the Ethical Committee of the University of Pisa (Approval number 83/3.0.7; Approval date 3 February 2007).

Twelve gelding thoroughbreds (aged 5.3 ± 1.6 years, mean \pm standard deviation [SD]) were enrolled after at least 2 months of rest following orthopaedic surgery (Table 1). Based on clinical examination and laboratory results, they were considered clinically healthy at the end of the 2-month resting period. All horses underwent an IV glucose tolerance test before the training period (IVGTT-pre), which was performed at 9 a.m. after at least 12 h of fasting. Two days later, the horses started a 1-month period of aquatraining. The pool was oval shaped and the direction of swimming was changed two to four times per swimming session, depending on the session length.

There was no water jet in the swimming pool. After the horses were familiarised with the swimming exercise, the protocol started with a daily 30 min swimming session during the first week. The duration of each session was then increased by 10 min every week up to 60 min in the last week, for the 1-month exercise period. The horses always swam with their usual rhythm and were never forced to increase their swim rate. The exercise intensity during each swimming session was monitored using a heart rate (HR) monitor (Polar RS800-CX) connected by a chest belt. Blood lactate was measured with a lactate meter (Accutrend Lactate, Micralab srl) 5 min before and after the last day of each training week. IVGTT (IVGTT-post) was repeated 2 days after the end of the training period using the same procedure as described for IVGTT-pre. The horses were weighed pre- and post-training, before performing each IVGTT. During the study, horses were fed according to the exercise activity and their body mass.

Two 14G IV catheters were inserted into the left and right jugular veins and the IVGTT was performed by administering 300 mg/kg of glucose IV (Tinworth et al., 2012) for 2 min through the right jugular catheter. Blood samples were collected from the left jugular catheter in lithium-heparin coated tubes 5 min before (basal value), and 5, 15, 25, 35, 45 and 60 min after glucose administration was completed (adapted from Firshman and Valberg, 2007). The catheters were flushed with 0.9% saline solution after each sampling to remove residual blood. To avoid *in vitro* glycolysis, plasma was separated within 15 min (Walton, 2013). Blood samples were centrifuged at 2.100 *g* for 10 min, and plasma was frozen at -18 °C and analysed in a single batch. Plasma glucose was measured using the enzymatic colorimetric method (Glucose SL, Assel Srl). Each sample was assayed in duplicate and intra-assay coefficients of variation (CVs) were $8.2 \pm 4.0\%$ across all samples. Plasma insulin was determined by radioimmunoassay (Coat-A-Count insulin, Siemens Medical Solution Diagnostics), which has previously been validated for use in horses (Tinworth et al., 2009) and in our laboratory. Each sample was assayed in duplicate and intra-assay CVs were $7.1 \pm 3.2\%$ across all samples. Peak glucose concentration (P_{gluc}) and glucose clearance rate (Cl) were

calculated using an add-in program for Microsoft Excel (PKSolver; Zhang et al., 2010). Area under the curve (AUC) was calculated for insulin (AUC_{ins}) and glucose (AUC_{gluc}) using the trapezoidal method (GraphPad Prism 6).

The Shapiro-Wilk test was performed to check data distribution. Paired t tests (GraphPad Prism 6, USA) were performed to evaluate differences in body mass, blood lactate, plasma glucose and insulin concentrations, AUC_{gluc} , AUC_{ins} , P_{gluc} and glucose clearance rate (CI glucose) in the pre- and post-training period. Statistical significance was set at $P < 0.05$.

During the last week of training, mean HR was < 180 beats/min (Fig. 1). No significant differences in blood lactate were determined between the pre- (1.9 ± 0.6 mmol/L) and post-training periods (2.1 ± 0.4 mmol/L; $P = 0.160$). Blood lactate remained below the threshold for accumulation (i.e. aerobic exercise) during the entire training period. There were no significant differences in body mass between the pre- (420.5 ± 17.3 kg) and post-training periods (412.5 ± 18.1 kg). Plasma glucose concentrations were higher in the pre-training period at 15 min ($P = 0.0001$), 25 min ($P = 0.0047$) and 35 min ($P = 0.0119$), than in the post-training period (Fig. 2). Basal plasma insulin was significantly lower at pre-training ($P = 0.0209$), and significantly higher at 45 min ($P = 0.0103$) and 60 min ($P = 0.0238$) than during the post-training period (Fig. 3). Pre-training AUC_{gluc} and P_{gluc} were significantly higher than post-training values ($P = 0.0275$ and 0.0430 , respectively); AUC_{ins} and glucose CI remained unchanged over the same period (Table 2).

GLUT-4 mediates glucose transport in muscle tissue under insulin control, and exercise stimulates the translocation of these proteins from the intracellular environment to the plasma membrane, facilitating the entry of glucose into the cell (Richter and Hargreaves, 2013). Moderate-intensity training in horses results in increased plasma glucose concentration during submaximal exercise, which is probably due to changes in plasma gluoregulatory hormones, such as insulin

(Geor et al., 2002). Moreover, short-term training increases tissue insulin sensitivity, GLUT-4 content and glycogen synthase activity in the skeletal muscle (Stewart-Hunt et al., 2006). Our study demonstrated that 1 month of submaximal aquatraining significantly changed equine metabolic response to an IVGTT. There was a slight increase in basal insulin and a more rapid decrease in insulin in the post-training period compared to the pre-training period. These changes, combined with lower glucose (AUC_{gluc} and P_{gluc}) in the post-training period, may explain more rapid changes in insulin-related glucose concentration in the post-training IVGTT.

It is accepted that insulin regulates glucose uptake in skeletal muscle in humans (Richter and Hargreaves, 2013), and in horses (Duehlmeier et al., 2010). Therefore, our results could be explained by enhanced insulin action and peripheral insulin sensitivity in the post-training period compared with pre-training values. To the authors' knowledge, this is the first published investigation of the effect of aquatraining exercise on plasma glucose and insulin values in horses. Our results highlight the need for further investigations in this area to understand the effects of low impact training on equine glucose metabolism.

Conflict of interest statement

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Figure legends

Fig. 1. Heart rate during a 60 min aquatraining session performed during the last week of training.

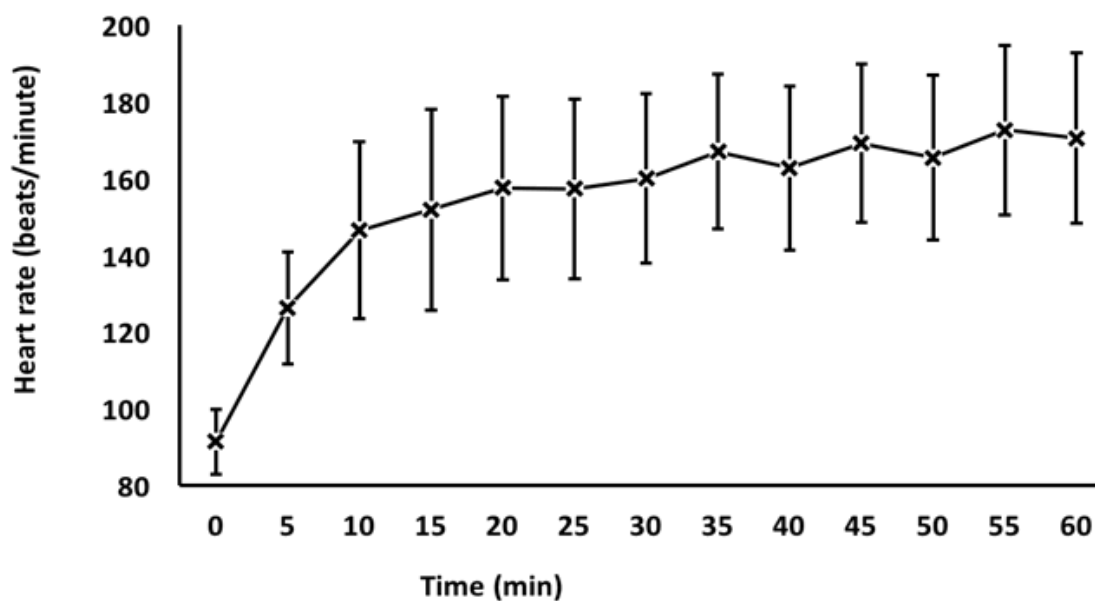
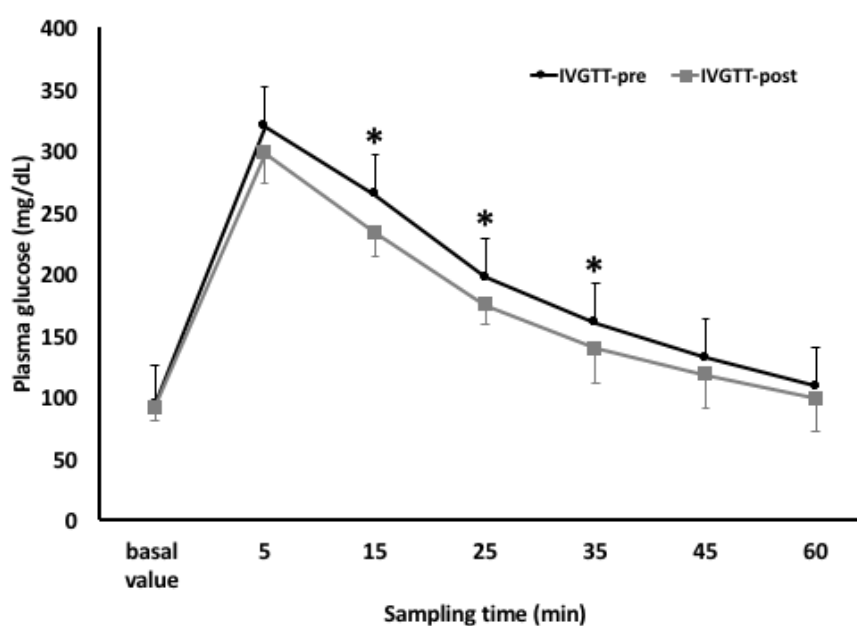
Fig. 2. Mean \pm standard deviation glucose values obtained after an IV glucose tolerance test (IVGTT) carried out before and after 1 month training in a swimming pool (* $P < 0.05$).

Fig. 3. Mean \pm standard deviation insulin values obtained after an IV glucose tolerance test (IVGTT) carried out before and after 1 month training in a swimming pool (* $P < 0.05$).

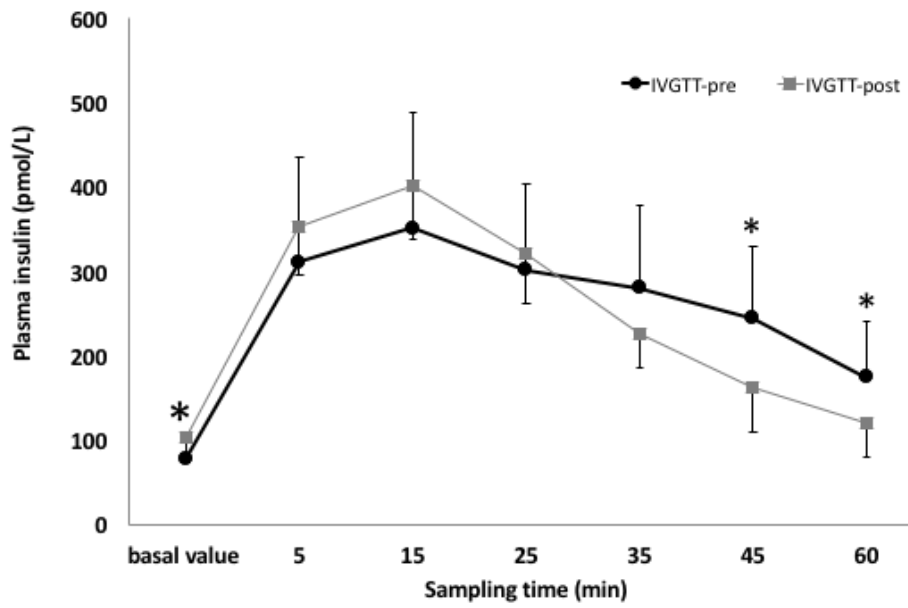


Table 1.

Orthopedic diagnoses in enrolled horses

Horse	Surgical procedure	Lesion
1	Arthroscopy	OCD right metacarpophalangeal joint
2	Tenoscopy	Desmitis of the annular ligament
3	Arthroscopy	OCD left metacarpophalangeal joint
4	Arthroscopy	OCD right and left metatarsophalangeal joints
5	Arthroscopy	OCD right metacarpophalangeal joint
6	Tenoscopy	DDFT chronic fibrosing synovitis
7	Arthroscopy	OCD right and left metacarpophalangeal joints
8	Arthroscopy	OCD right femoropatellar joint
9	Arthroscopy	SBCs proximal cranial right tibia
10	Tenoscopy	SDFT tear of the manica flexoria
11	Arthroscopy	OCD right femoropatellar joint
12	Arthroscopy	OCD left metacarpophalangeal joint

OCD, Osteochondritis dissecans; DDFT, Deep digital flexor tendon; SBCs, Subchondral bone cysts; SDFT, Superficial digital flexor tendon.

Table 2.

Area under the curve for glucose (AUC_{gluc}) and insulin (AUC_{ins}), peak glucose concentration (P_{gluc}), and glucose clearance rate (CI glucose) for the pre- (PRE) and post-training period (POST), with superscripts within each row indicating significant differences.

Parameter	PRE	POST	<i>P</i>
AUC_{gluc} (0 to 60 min) g/dL*min	11289 ± 1233 ^a	10118 ± 927 ^b	0.0275
P_{gluc} (g/dL)	324.30 ± 31.04 ^a	299.20 ± 24.21 ^b	0.0430
CI glucose (dL/min)	0.098 ± 0.023	0.011 ± 0.026	0.2850
AUC_{ins} (pmol/L*min)	16185 ± 5601	15315 ± 1766	0.6130