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Insulin signaling in various equine tissues under basal conditions and acute stimulation by intravenously injected insulin

Tobias Warnken [a,b,*], Ralph Brehm [c], Karsten Feige [a], Korinna Huber [d]

[a] Clinic for Horses, University of Veterinary Medicine Hannover, Foundation, Bünteweg 9, 30559 Hannover, Germany
[b] Department of Physiology, University of Veterinary Medicine Hannover, Foundation, Bischofsholer Damm 15, 30173 Hannover, Germany
[c] Institute of Anatomy, University of Veterinary Medicine Hannover, Foundation, Bischofsholer Damm 15, 30173 Hannover, Germany
[d] Institute of Animal Science, Faculty of Agricultural Sciences, University of Hohenheim, Fruwirthstraße 35, 70599 Stuttgart, Germany

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ABSTRACT

The aim of the study was to analyze key proteins of the equine insulin signaling cascade and their extent of phosphorylation in biopsies from muscle tissue (MT), liver tissue (LT), and nuchal AT, subcutaneous AT, and retroperitoneal adipose tissues. This was investigated under unstimulated (B1) and intravenously insulin stimulated (B2) conditions, which were achieved by injection of insulin (0.1 IU/kg bodyweight) and glucose (150 mg/kg bodyweight). Twelve warmblood horses aged 15 ± 6.8 yr (yr), weighing 559 ± 79 kg, and with a mean body condition score of 4.7 ± 1.5 were included in the study. Key proteins of the insulin signaling cascade were semiquantitatively determined using Western blotting. Furthermore, modulation of the cascade was assessed. The basal expression of the proteins was only slightly influenced during the experimental period. Insulin induced a high extent of phosphorylation of insulin receptor in LT ($P < 0.01$) but not in MT. Protein kinase B and mechanistic target of rapamycin expressed a higher extent of phosphorylation in all tissues in B2 biopsies. Adenosine monophosphate protein kinase, as a component related to insulin signaling, expressed enhanced phosphorylation in MT ($P < 0.05$) and adipose tissues (nuchal AT $P < 0.05$; SCAT $P < 0.01$; retroperitoneal adipose tissue $P < 0.05$), but not in LT at B2. Tissue-specific variations in the acute response of insulin signaling to intravenously injected insulin were observed. In conclusion, insulin sensitivity in healthy horses is based on a complex concerted action of different tissues by their variations in the molecular response to insulin.

1. Introduction

To date insulin action on insulin-sensitive tissues is not well studied in horses. In all mammalian species, insulin-dependent tissues are liver, skeletal muscle, and the adipose tissues. Insulin binds to its specific receptor (InsR) which activates the InsR substrate and the downstream signaling cascade (Fig. 1). A central hub of this signaling cascade is the protein kinase B (PKB/AKT) which is activated by phosphorylation. From there, the protein synthesis pathway is positively influenced by insulin. This is reflected by enhanced phosphorylation of mechanistic target of rapamycin (mTOR), a serine-threonine kinase which stimulates translation initiation. PKB/AKT inhibits lipolysis by activating phosphodiesterase 3 which inhibits cAMP production by protein kinase A and thereby reduces phosphorylation of hormone-sensitive lipase (HSL) and its lipolytic activity. Furthermore, insulin stimulates

* Corresponding author. Tel.: +49 511-953-6682; fax: +49 511-953-6510.
E-mail address: tobias.warnken@tiho-hannover.de (T. Warnken).

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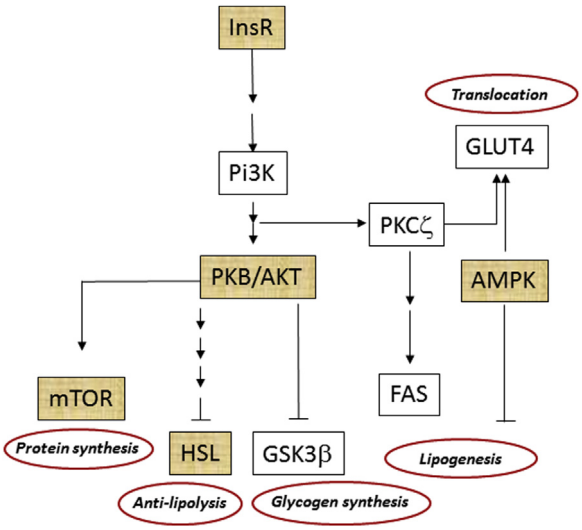

Fig. 1. Key components of insulin signaling examined in horses of protein level (according to http://www.genome.jp/kegg/pathway.html). All proteins highlighted in color were examined on their total protein content and their extent of phosphorylation under unstimulated basal conditions (B1) and under stimulated conditions (B2) with provoked hyperinsulinemia and hyperglycemia in equine adipose tissues, liver tissue, and muscle tissue. Arrows indicate activating effects and horizontal lines indicate inhibiting effects. InsR, insulin receptor; Pi3K, phosphoinositide 3 kinase; PKB/Akt, protein kinase B; mTOR, mechanistic target of rapamycin; HSL, hormone-sensitive lipase; GSK-3β, glycogen synthase kinase 3β; FAS, fatty acid synthase; PKC ζ, protein kinase C ζ; AMPK-α, adenosine monophosphate–activated kinase α; GLUT4, glucose transporter 4. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

lipogenesis by increasing fatty acid synthase (FAS) expression and activity, a pathway inhibited by adenosine monophosphate-activated kinase (AMPK). The glucose transporter 4 (GLUT4) translocation from cytosol stores into plasma membranes is stimulated by atypical protein kinase C zeta (PKC ζ) resulting in increased glucose uptake by insulin-dependent tissues. Insulin-regulated glycogen synthase kinase 3 beta (GSK3β) is a critical enzyme regulating glucose storage of cells [1]. Only few studies have been done in horses to detect components of insulin signaling on protein level and to evaluate their modulation by insulin. In equine cardiac and skeletal muscle, InsR and insulin-like growth factor 1 receptor protein expression was studied in healthy horses, revealing no differences between the muscle types [2]. However, on mRNA level, hyperinsulinemic horses expressed higher mRNA amounts of PKB/AKT, GSK3β, GLUT1, and GLUT4 in the cardiac, but not in the skeletal muscle [2]. Although it is difficult to interpret what functional consequences the changes in mRNA expression may have, the tissue-dependent sensitivity to high insulin concentrations appeared to be a physiological feature in horses. Hyperinsulinemia established by clamp procedures provoked an increase in the extent of phosphorylation in PKB/AKT, 4E binding protein 1, and riboprotein S6. The latter 2 proteins are downstream targets of mTOR in the gluteus medius muscle [3]. This indicates an insulin-driven stimulation of protein synthesis in this muscle in horses. Muscle GLUT4 expression and -translocation to the plasma membrane appeared not to be dependent on insulin, however insulin-resistant horses had lower GLUT4 in the plasma membrane of muscle cells [4]. To understand the regulation of insulin-dependent pathways it is necessary to increase the knowledge on physiological features of the insulin signaling pathway in horses. Therefore, the objectives of this study were to determine the protein expression of key components of insulin signaling and their extent of phosphorylation in the main peripheral insulin-sensitive tissues in horses under basal conditions and under hyperinsulinemic and hyperglycemic conditions provoked by injection of insulin and glucose.

2. Materials and methods

2.1. Animals

This study was approved by the ethics committee of the University of Veterinary Medicine, Hannover, and the Lower Saxony State Office for Consumer Protection and Food Safety, in accordance with the German Animal Welfare Law (File No. 33.14 42502-04-13/1259). The experiment was conducted at the Clinic for Horses, University of Veterinary Medicine Hannover, Germany. Twelve healthy warmblood breed horses were included in the study (Table 1). There were six mares, three geldings, and three stallions aged from 4 to 24 yr (15 ± 6.8 yr) and weighing 440 to 695 kg (559 ± 79 kg). The body condition score (BCS) of the horses covered a wide range from 1.9 to 7.5 with a mean BCS of 4.7 ± 1.5. The BCS was determined as the average of five independent assessors according to the scoring system of Henneke et al [5]. Starting 2 wk before the beginning of the experiment, the horses were fed average mixed-grass hay on maintenance requirement twice daily and no supplementary feeding was provided. They were stabled in individual boxes, under standardized feeding and management conditions and were not exercised, except for 2 h access to a large paddock, daily. All horses underwent clinical examinations and laboratory screenings before beginning the study. To exclude horses with insulin dysregulation (ID), all horses underwent an oral glucose testing procedure with 1 g/kg BW glucose administered via nasogastric tubing [6]. Furthermore,

Table 1
Horses background data.

Horse	Age (yr)	Sex	BW (kg)	Body condition score (BCS)
1	11	Gelding	650	5.3
2	19	Mare	465	2.9
3	19	Mare	440	1.9
4	24	Gelding	480	3.0
5	4	Stallion	540	6.0
6	15	Mare	619	5.1
7	15	Stallion	515	5.1
8	25	Mare	570	4.5
9	4	Stallion	560	4.0
10	11	Gelding	640	5.9
11	20	Mare	537	4.7
12	13	Mare	695	7.5

Abbreviation: BW, bodyweight.
Animals background data.