Effect of blood lactate-guided conditioning of horses with exercises of differing durations and intensities on heart rate and biochemical blood variables

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ABSTRACT: The velocity at which blood lactate concentration ([LA]) of 4 mmol/L is reached (v4) is widely used to determine fitness, but there are few published data on using [LA] as a guide for the exercise speed for training in horses. In this study, the effect of 3 conditioning programs with [LA] guided exercise speeds on v4, v200 (speed at a heart rate of 200 beats/min), blood [LA], plasma FFA ([FFA]), and alanine concentrations ([alanine]), before and after exercise, as well as heart rate during exercise, of horses was examined. Six 2-yr-old Haflinger stallions underwent an initial treadmill-based standard exercise test (SET). A regression analysis [LA]-speed relationship was used to calculate v1.5, v2.5, and v4. Horses were then randomly assigned to 1 of 3 conditioning programs according to a 3 × 6 Latin square design. During 6 wk, horses exercised on the treadmill every other day for 45 min at their calculated v1.5 or v2.5 or 25 min at their v4. Each conditioning period (CDP) was followed by 5 wk without conditioning. At 2 and 9 d, and 5 wk, after the end of the CDP, the horses performed another SET to evaluate again the v4 and v200. Blood [LA], plasma [FFA], and [alanine] were measured before and after heart rate during exercise sessions 1, 11, and 21 in each CDP. None of the exercise programs had an effect on v4 and v200 (P > 0.05). The increase of the mean [LA] after exercise decreased during CDP (P < 0.05), and the increase of mean heart rate during exercise tended to decrease as well (P = 0.07). There was no difference among the conditioning programs. Plasma [FFA] before exercise was not influenced by the CDP (P > 0.05). The plasma [FFA] was always greater after exercise (P < 0.05), but there was no difference among conditioning programs. Overall, the increase was greatest after the 21st exercise session compared with the 1st and 11th exercise sessions (P < 0.05). The mean plasma [alanine] before exercise remained similar during all CDP (P > 0.05). Mean plasma [alanine] of the horses was increased after all exercise sessions measured (P < 0.05). There was no difference among conditioning programs (P > 0.05). It is concluded that conditioning with the exercise types used had small effects. This could have been because the exercise stress was too small, but also because the workload was not increased during the CDP.

Key words: alanine, blood, conditioning, exercise, horse, lactate

INTRODUCTION

Blood lactate concentrations ([LA]) are regularly used to assess the level of fitness in the sport horse. The term vLA is used to represent the velocity at which the [LA] in the blood reaches a certain value (i.e., v1.5 is the velocity that elicits a [LA] of 1.5 mmol/L). One quantitative variable of the blood [LA]-running speed relation, v4 (velocity at which the [LA] of 4 mmol/L is reached), is a repeatable and reliable value for the determination of fitness and competitive success in horses (Lindner, 2000). It is also well documented that v4 increases with training in horses (von Wittke et al., 1994; Eaton et al., 1999), with the magnitude of increase being dependent upon factors such as the intensity, dura-
tion, and frequency of exercise. However, studies on the comparison of the effects of these variables of exercise are scarce (Werkmann et al., 1996; Gansen et al., 1999; Rivera et al., 2007).

Biochemical variables are measured in blood or plasma to describe the contribution of the different energy pathways and fuel sources to supply the required energy during exercise. This is especially true for the horse because its size almost prohibits the use of radioactive tracer methods to study its metabolism. The measurement in blood of [LA] and the concentrations of FFA ([FFA]) and alanine ([alanine]) as variables of carbohydrate, fat, and protein metabolism, respectively, is therefore an attempt to increase knowledge on the involvement of these fuels in equine exercise physiology (McMiken, 1983).

Our laboratory is interested in the effect of blood lactate-guided exercise for conditioning of horses. Previous studies showed that exercise at the lesser intensities (v1.5 to v2.5) for 45 min was more effective at increasing glycogen concentration in muscle than exercise at the greater intensity (v4) and 25-min duration (Gansen et al., 1999). This article describes whether the exercise at lesser blood [LA] derived speeds for a longer period of time was also more effective at increasing v4 and v200 than exercise of shorter duration but at greater blood [LA] derived speeds. In addition, the effect of the conditioning programs on plasma [FFA] and [alanine] is presented.

**MATERIALS AND METHODS**

This experiment was approved by the Animal Welfare Committee of the State of North-Rhine-Westfalia, Germany.

**Horses**

Six 2-yr-old Haflinger stallions participated in the current study; the horses were housed in stables. The daily feeding ration consisted of 5 kg of concentrate and 0.2 kg of mineral supplement. In addition, during early summer, horses received 3 kg of hay and 16 kg of grass; during late summer, they were given grass only, and during the winter, 6 kg of hay. Water and straw were available ad libitum. The mean BW of the horses was 406 ± 23.6 kg at the beginning and 430 ± 23.8 kg at the end of the study.

**Study Design**

Three different conditioning programs were examined, and all horses underwent all 3 programs. Before the first conditioning period (CDP), horses were randomly assigned to 1 of the 3 conditioning programs. Each CDP lasted for 6 wk. After a CDP and after completing a resting period of 5 wk, horses were switched to another conditioning program according to a 3 × 6 Latin square arrangement of treatments (3 treatments, 6 horses). The total duration of the experiment was 33 wk without counting the adaptation period before starting the study of additional 6 wk.

**Exercise**

All exercise was conducted on a treadmill at an incline of 17% (during the resting period, 0%; Jünck Konditrainer, Borken, Germany). The maximal speed at which the treadmill could operate was 18 km/h (5 m/s). In the pretrial period, it became evident that at this maximal speed it was not possible to obtain a [LA] of 4 mmol/L in the horses during the standardized exercise test (SET) with reasonable duration of exercise when they were not worked at the 17% slope. This is why it was opted to exercise the horses in such way. Lameness did not develop during the experiment. Although locomotion was different than with no or less incline, no effects on the variables measured were expected.

**Exercise for Conditioning**

During a CDP, horses were exercised every other day for 6 wk (21 exercise sessions within 1 CDP). The exercise was for 45 min at their individual v1.5, v2.5, or v4. On the days between the exercise sessions, horses were kept in a large paddock. When weather did not allow for outside paddock housing, horses were walked for 30 min on a horse walker.

**Exercise During the Resting Period**

The exercise during the 5 wk before starting the next CDP consisted of 1 h walking on a horse walker on 3 d per week and treadmill exercise on 2 d per wk (5 min at 2.2 m/s and 15 min at 2.8 m/s, 0% incline).

**SET**

The velocity v1.5, v2.5, and v4 at which each horse was exercised were calculated individually by exponential equation using the blood [LA]-running speed relationship of the horse obtained with a SET. The SET was performed by each horse before each CDP, 2 and 9 d, as well as 5 wk, after finishing each CDP; v1.5, v2.5, and v4 are defined as the speed at which mathematically a blood [LA] of 1.5, 2.5, or 4 mmol/L was determined when run under the defined conditions.

The SET consisted of several steps of 5-min duration each. Between 2 consecutive steps, there was a resting period of 60 s. During this time, blood was sampled to determine the [LA] with a dry chemistry device (Accusport, Boehringer Mannheim, Germany). The speed in the first step was 2.8 m/s and was increased in each consecutive step by 0.3 m/s. The test was finished when the blood [LA] of the horses reached 4 mmol/L or above.
During the test, horses were fitted with a commercial heart rate meter and their heart rate recorded. The speed at which heart rate reached 200 beats/min (v200) was calculated through linear regression from the heart rate-running speed relation.

**Clinical Variables**

Heart rate was monitored continuously during every second exercise session and all SET with a heart rate meter (Polar Sport Tester, Kempele, Finland) attached to the thorax of the horse. Body weight was determined by weighing the horses on a scale before and after exercise sessions 1, 11, and 21. The accuracy of the scale was ±100 g. Body temperature was measured rectally with a commercially available thermometer before and after exercise sessions 1, 11, and 21. The accuracy of the thermometer was ±0.1°C.

**Biochemical Variables**

Five to seven milliliters of blood were taken by jugular venipuncture into Na-heparinized, evacuated tubes. For lactate analysis, this was done immediately after each step during the SET and at the end of the exercise session 1, 11, and 21 in each CDP. For measuring plasma [FFA] and [alanine], blood samples were taken before and after the exercise session 1, 11, and 21 in each CDP.

On site blood [LA] analysis was done using the dry chemistry device (Accusport). This machine provided the results within 1 min of starting the analysis and was used to decide on when to stop the SET ([LA] above 4 mmol/L). In addition, 20 µL of each blood sample was immediately collected with a disposable capillary pipette and transferred to vials with 200 µL of ice-cold 0.6 N perchloric acid. These vials were centrifuged at 12,000 × g for 5 min at environmental temperature ranging between 25 and 10°C. The supernatant was transferred to another vial and kept stored at −20°C until analysis. Analysis was carried out with an EPOS 5060 lactate analyzer (Eppendorf, Wesseling, Germany) using an enzymatic test kit (Behring OSUA 40, Marburg, Germany). These lactate values of the horses were used to calculate the parameters of the blood lactate-running speed relation v1.5, v2.5, and v4 by exponential equation. These values were used to guide the speed of the exercise in the CDP.

For [FFA] and [alanine] analysis, blood samples were centrifuged at 5,000 × g for 10 min at environmental temperature ranging between 25 and 10°C. Plasma was then transferred to vials and stored at −20°C until analysis within 4 wk. Analysis of [FFA] was performed using a colorimetric assay according to Itaya (1977). The staining reagent was a mixture of diphenylcarbacide (5:95). Palmitic acid dissolved in chloroform (400 µmol/L) served as standard. Alanine was measured enzymatically according to Williamson (1985).

**Statistical Analysis**

Data are presented as mean and SD. For [FFA] and [alanine], the percentile increase after exercise was calculated. All values were analyzed with ANOVA for repeated measures to examine the effects of conditioning. In those cases where significant effects were found, a Fisher’s test was used as post-hoc test with P < 0.05 denoting significant differences.

**RESULTS**

**General**

Mean speed of horses during exercise was 3.1, 3.4, and 3.8 m/s for v1.5, v2.5, and v4, respectively. The [LA] after exercise ranged from 1.60 to 6.70 mmol/L after 45 min of exercise at v1.5, 1.35 to 6.85 mmol/L after v2.5, and 2.15 to 8.45 mmol/L after 25 min of exercise at v4.

The mean heart rate during exercise ranged between 148 and 176, 153 and 185, and 165 and 195 beats/min after v1.5, v2.5, and v4, respectively. The conditioning programs used did not impair health of horses. Rectal temperature increased after exercise regardless of its type on average by 2.84°C (±0.58; P < 0.05). The largest decrease of BW was measured after exercise at v2.5 during 45 min (10.8 ± 3.0 kg) and the smallest after exercise at v4 during 25 min (6.44 ± 3.0 kg; for v1.5 during 45 min it was 9.57 ± 2.26 kg). The effect was larger for exercise at v2.5 during 45 min than for exercise at v4 during 25 min only (P < 0.05).

**v4, v200, [LA] After, and Heart Rate During, Exercise Sessions**

None of the exercise programs had any effect on v4 or v200 (P > 0.05). The largest mean increase of v4 after conditioning was calculated when horses were exercised during 45 min at their v1.5 (on average by 7.3% compared with 4.9 and 2.3% for exercise at v2.5 for 45 min and v4 for 25 min, respectively). For v200, the largest increase was calculated in the horses exercising at their v1.5 during 45 min (9.6%), followed by exercise at v4 during 25 min (6.9%) and at v2.5 during 45 min (6.4%). During the 5 wk of the resting period, the v4 tended to decrease after the CDP where the horses were exercised at their v1.5 for 45 min and v4 for 25 min, and they remained constant after the CDP with exercise at v2.5 during 45 min; v200 did not change during the resting periods.

The mean blood [LA] after exercise differed between exercise types (P < 0.001; 3.43 ± 1.62 mmol/L, 4.60 ± 1.75 mmol/L, and 4.84 ± 1.50 mmol/L after exercise at v1.5 and v2.5 during 45 min and v4 during 25 min, respectively). The mean heart rate during exercise also differed between the exercise types used (P < 0.001; 164 ± 7, 173 ± 8, and 180 ± 10 beats/min during exercise at v1.5 and v2.5 during 45 min and v4 during 25 min, respectively). The increase of the mean [LA] after exer-
Crise decreased during CDP ($P < 0.05$; Table 1) and the increase of mean heart rate during exercise tended to be smaller as well ($P = 0.07$; Table 2), although there was no difference among the conditioning programs.

**FFA**

Plasma [FFA] before exercise was not influenced by the conditioning programs. The same was true for the percent increases of plasma [FFA] after exercise ($P > 0.05$; Table 3). Mean plasma [FFA] was increased after all exercise sessions ($P < 0.05$). For all CDP together, the percent increase was larger in the 21st exercise session than in the 1st ($P < 0.05$).

**Alanine**

Conditioning did not have an effect on plasma [alanine] of horses before exercise ($P < 0.05$). Mean plasma [alanine] of the horses was increased after all exercise sessions ($P < 0.05$; Table 4). There was no effect of conditioning on the percent increase of alanine due to exercise ($P > 0.05$). Overall the increase of mean plasma [alanine] was greater in the 11th exercise session than in the 1st and in the 21st exercise session ($P \leq 0.05$).

**DISCUSSION**

The effects of the different conditioning programs used to exercise the horses on $v_4$, $v_{200}$, [LA] after exercise, and heart rate during exercise, although sometimes reaching the level of significance, were quite small. The reason for this could be that the different exercise types were not stressful enough to induce larger changes in these variables. However, conditioning programs with exercise of 45-min duration at $v_{1.5}$ and $v_{2.5}$ increased the glycogen concentration in the M. gluteus medius (Ganssen et al., 1999). This became evident 9 d after finishing the CDP with these 2 conditioning programs. Also, total area of mitochondria and of myofibrils, as well as their number in muscle, were changed more by these CDP (Dag, 1998). But these changes were measured 2 d after finishing the CDP, whereas at the same time, exercise decreased during CDP ($P < 0.05$; Table 1) and the increase of mean heart rate during exercise tended to be smaller as well ($P = 0.07$; Table 2), although there was no difference among the conditioning programs.

### Table 1. Lactate concentration in blood of horses after exercise during different conditioning programs (6 horses; mean ± SD; mmol/L)

<table>
<thead>
<tr>
<th>Exercise session</th>
<th>Conditioning program&lt;sub&gt;1&lt;/sub&gt;</th>
<th>45 min at $v_{1.5}$</th>
<th>45 min at $v_{2.5}$</th>
<th>25 min at $v_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td></td>
<td>3.32 ± 1.03</td>
<td>4.63 ± 2.12</td>
<td>4.93 ± 1.24</td>
</tr>
<tr>
<td>3rd</td>
<td></td>
<td>2.43 ± 0.66</td>
<td>3.67 ± 1.70</td>
<td>4.26 ± 1.36</td>
</tr>
<tr>
<td>5th</td>
<td></td>
<td>3.32 ± 1.27</td>
<td>4.78 ± 1.48</td>
<td>5.03 ± 0.70</td>
</tr>
<tr>
<td>7th</td>
<td></td>
<td>3.91 ± 2.13</td>
<td>5.38 ± 2.22</td>
<td>4.91 ± 2.07</td>
</tr>
<tr>
<td>9th</td>
<td></td>
<td>3.49 ± 1.63</td>
<td>5.53 ± 1.87</td>
<td>5.43 ± 1.34</td>
</tr>
<tr>
<td>11th</td>
<td></td>
<td>3.80 ± 1.74</td>
<td>5.62 ± 1.30</td>
<td>5.87 ± 1.37</td>
</tr>
<tr>
<td>13th</td>
<td></td>
<td>3.16 ± 1.62</td>
<td>3.83 ± 1.06</td>
<td>4.19 ± 1.10</td>
</tr>
<tr>
<td>15th</td>
<td></td>
<td>3.57 ± 2.40</td>
<td>4.31 ± 1.72</td>
<td>4.20 ± 1.98</td>
</tr>
<tr>
<td>17th</td>
<td></td>
<td>4.22 ± 2.43</td>
<td>4.84 ± 2.26</td>
<td>5.07 ± 1.77</td>
</tr>
<tr>
<td>19th</td>
<td></td>
<td>3.50 ± 1.31</td>
<td>4.15 ± 1.65</td>
<td>4.98 ± 1.40</td>
</tr>
<tr>
<td>21st</td>
<td></td>
<td>3.34 ± 1.82</td>
<td>4.04 ± 0.93</td>
<td>4.77 ± 2.49</td>
</tr>
</tbody>
</table>

<sup>1</sup>$v_{1.5}$, $v_{2.5}$, and $v_4$ = the velocity at which the lactate concentration in the blood reaches 1.5, 2.5, and 4 mmol/L, respectively.

### Table 2. Heart rate of horses during exercise during different conditioning programs (6 horses; mean ± SD; beats/min)

<table>
<thead>
<tr>
<th>Exercise session</th>
<th>Conditioning program&lt;sub&gt;1&lt;/sub&gt;</th>
<th>45 min at $v_{1.5}$</th>
<th>45 min at $v_{2.5}$</th>
<th>25 min at $v_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td></td>
<td>166 ± 7</td>
<td>172 ± 10</td>
<td>180 ± 13</td>
</tr>
<tr>
<td>3rd</td>
<td></td>
<td>162 ± 5</td>
<td>173 ± 12</td>
<td>181 ± 9</td>
</tr>
<tr>
<td>5th</td>
<td></td>
<td>165 ± 6</td>
<td>177 ± 9</td>
<td>186 ± 14</td>
</tr>
<tr>
<td>7th</td>
<td></td>
<td>165 ± 6</td>
<td>171 ± 8</td>
<td>178 ± 15</td>
</tr>
<tr>
<td>9th</td>
<td></td>
<td>165 ± 7</td>
<td>171 ± 5</td>
<td>185 ± 5</td>
</tr>
<tr>
<td>11th</td>
<td></td>
<td>166 ± 7</td>
<td>176 ± 6</td>
<td>180 ± 9</td>
</tr>
<tr>
<td>13th</td>
<td></td>
<td>164 ± 5</td>
<td>174 ± 6</td>
<td>178 ± 12</td>
</tr>
<tr>
<td>15th</td>
<td></td>
<td>164 ± 8</td>
<td>176 ± 5</td>
<td>178 ± 8</td>
</tr>
<tr>
<td>17th</td>
<td></td>
<td>163 ± 6</td>
<td>173 ± 9</td>
<td>181 ± 11</td>
</tr>
<tr>
<td>19th</td>
<td></td>
<td>161 ± 7</td>
<td>169 ± 5</td>
<td>178 ± 6</td>
</tr>
<tr>
<td>21st</td>
<td></td>
<td>161 ± 9</td>
<td>173 ± 7</td>
<td>178 ± 8</td>
</tr>
</tbody>
</table>

<sup>1</sup>$v_{1.5}$, $v_{2.5}$, and $v_4$ = the velocity at which the lactate concentration in the blood reaches 1.5, 2.5, and 4 mmol/L, respectively.
no change of the glycogen concentration was evident. This illustrates that the kinetics of adaptation of different variables to conditioning differ, assuming there are some. Thus, sometimes effects can be missed when the number of samples and measurements taken after a treatment is too small or the timing is wrong. In some studies on horses, increases of different muscle enzyme activities have been observed only after weeks of finishing a CDP (Guy and Snow, 1977; Snow and Guy, 1979; Foreman et al., 1990). Banister et al. (1996) reviewed the “off” metabolic stimulus signal after finishing a period of conditioning with adequate adaptations. This off-stimulus seems to produce a phase-lagged, cellular protein overshoot for up to several weeks after completion of a stimulating conditioning regime. Through this mechanism, the kinetics of adaptation of tissues and metabolism is a continuous process of which the duration and maximum very likely are related to the type of conditioning program. This hypothesis, and also the possibility of the need of different timings for optimal sampling the various variables, provides explanations for the distinct behavior of the variables measured in the horses of this study.

Another reason for the lack of larger effects could be that the speed of exercise during the CDP was the same for each horse, possibly not allowing for a larger adaptation of the variables of fitness determined. This assumption is supported by the study done by Trilk et al. (2002). They worked horses at their \( v_4 \) for 45 min, but adapting the speed of exercise every 2 wk according to the results of a SET. The \( v_2 \) increased every 2 wk; therefore, every 2 wk the horses were exercised at greater speeds. In total those horses increased their \( v_4 \) for up to 17% and their \( v_{180} \) for up to 16.5% during the 6 wk of conditioning.

In this study, the plasma [FFA] of horses before exercise was always below 200 µmol/L. The plasma [FFA] of horses under resting conditions varies greatly, ranging between 20 and 500 µmol/L (Snow et al., 1979; Gill et al., 1987; Orme et al., 1994, 1995; Hyyppä et al., 1997). Orme et al. (1994) reported a 4.5-fold increase of the mean plasma [FFA] of horses between 0400 and 1000 h. In addition, food deprivation produces marked increases of plasma [FFA] (Zimmerman et al., 1992).

The [FFA] in plasma of the horses increased after all exercise types used with no differences among the conditions. The effort induced by the different durations and intensities of the 3 types of exercise appears to have been too similar to induce differential effects. In addition, the response to exercise of plasma [FFA] varied very much, which is due to the low number of horses, and may have limited detecting changes. From the literature, it is known that the plasma [FFA] in general responds to exercise with an increase of its values. Long-lasting exercise, such as endurance riding, induces greater plasma [FFA] in horses (Lucke and Hall, 1980a,b; Hambitzer and Bent, 1988; Sloet van Oldruitenborgh-Oosterbaan et al., 1991) than exercise of short duration but near-maximal intensity (Snow et al., 1979; Pösö et al., 1989).

Table 3. Free fatty acids concentration in plasma of horses before and after exercise during different conditioning programs (6 horses; mean ± SD; µmol/L)

<table>
<thead>
<tr>
<th>Exercise session</th>
<th>Time of blood sampling</th>
<th>Conditioning program</th>
<th>45 min at ( v_{1.5} )</th>
<th>45 min at ( v_{2.5} )</th>
<th>25 min at ( v_4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Before</td>
<td>98 ± 62</td>
<td>99 ± 90</td>
<td>92 ± 80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>578 ± 355</td>
<td>642 ± 352</td>
<td>321 ± 138</td>
<td></td>
</tr>
<tr>
<td>11th</td>
<td>Before</td>
<td>92 ± 59</td>
<td>90 ± 47</td>
<td>96 ± 52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>948 ± 423</td>
<td>847 ± 515</td>
<td>442 ± 121</td>
<td></td>
</tr>
<tr>
<td>21st</td>
<td>Before</td>
<td>78 ± 33</td>
<td>64 ± 44</td>
<td>41 ± 26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>715 ± 310</td>
<td>673 ± 437</td>
<td>308 ± 157</td>
<td></td>
</tr>
</tbody>
</table>

\( v_{1.5}, v_{2.5}, \) and \( v_4 \) = the velocity at which the lactate concentration in the blood reaches 1.5, 2.5, and 4 mmol/L, respectively.

Table 4. Alanine concentration in plasma of horses before and after exercise during different conditioning programs (6 horses; mean ± SD; µmol/L)

<table>
<thead>
<tr>
<th>Exercise session</th>
<th>Time of blood sampling</th>
<th>Conditioning program</th>
<th>45 min at ( v_{1.5} )</th>
<th>45 min at ( v_{2.5} )</th>
<th>25 min at ( v_4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Before</td>
<td>254 ± 57</td>
<td>270 ± 85</td>
<td>289 ± 48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>407 ± 80</td>
<td>441 ± 110</td>
<td>459 ± 82</td>
<td></td>
</tr>
<tr>
<td>11th</td>
<td>Before</td>
<td>264 ± 46</td>
<td>265 ± 27</td>
<td>251 ± 31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>439 ± 41</td>
<td>490 ± 98</td>
<td>470 ± 55</td>
<td></td>
</tr>
<tr>
<td>21st</td>
<td>Before</td>
<td>283 ± 59</td>
<td>289 ± 49</td>
<td>276 ± 43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>412 ± 42</td>
<td>472 ± 75</td>
<td>453 ± 83</td>
<td></td>
</tr>
</tbody>
</table>

\( v_{1.5}, v_{2.5}, \) and \( v_4 \) = the velocity at which the lactate concentration in the blood reaches 1.5, 2.5, and 4 mmol/L, respectively.
Pooling the data of all 3 conditioning programs demonstrated a greater percentage increase of plasma [FFA] after the 21st exercise session than after the 1st and the 11th exercise sessions. A greater increase after exercise of plasma [FFA] in Andalusian horses conditioned for 6 mo was described by Muñoz et al. (2002). Those authors attributed the increase to increased oxygen uptake after conditioning based on decreased plasma [LA] after exercise. The plasma [FFA] after exercise of Angloarabian and Arabian breed horses submitted to the same conditioning program did not change. The different results may have been because the Andalusian horses started with less level of fitness than the horses of the other breeds, based on the reaction of the plasma [LA], [FFA], glucose concentrations, and CK activities to exercise. Exactly the opposite behavior of plasma [FFA] after exercise was observed by Sloet van Oldruitenborgh-Oosterbaan et al. (1990) in Dutch Warmblood stallions conditioned for 100 d and Watermülder (2002) in Standardbreds trained for 4 mo, a smaller increase of [FFA]. In both studies, the heart rate during and after exercise as well as the plasma [LA] after exercise were less, indicating improved fitness. Conditioning programs applied in these studies differ widely and may account for the differing results. Explanations for either behavior can be given. It is well documented that endurance activities increase the energy utilization from fat while sparing carbohydrate sources and that at the same absolute intensity, fat oxidation contributes more to energy expenditure in trained endurance athletes compared with untrained men (Ranallo and Rhodes 1998; van Loon et al., 1999; Horowitz and Klein, 2000). In very few of the studies done in men on the contribution of FFA to the energy metabolism during and after exercise was the plasma [FFA] behavior also measured. Those reports that were found describe decreased [FFA] in response to endurance training (Martin et al., 1993; van Loon et al., 1999; Jacobs et al., 2006).

The majority of studies demonstrate that horses under resting conditions have a plasma [alanine] of less than 250 µmol/L (Lucke and Hall, 1980a; McKeever et al., 1986; Pösö et al., 1987, 1991). Only Jahn et al. (1991) measured a mean plasma [alanine] of 350 µmol/L. In this study, the range was 150 µmol/L to 350 µmol/L.

The plasma [alanine] after exercise was always greater than before exercise. Differences between conditioning programs were not found, although there was a tendency toward smaller increases during exercise at v1.5 for 45 min than during the other 2 conditioning programs, indicating that the increase of plasma [alanine] may be dependent on speed as well as duration of exercise. The largest increase for all conditioning programs became evident in the 11th exercise session. There was no further effect, which may be explained by the fact that the intensity of exercise was not increased further.

There are not many studies in horses on the behavior of plasma [alanine] during and after different exercise types. Based on studies found in the literature, with increasing intensity of exercise, an elevation of plasma [alanine] is observed (Pösö et al., 1987, 1991; Miller and Lawrence, 1988; Jahn et al., 1991; Watermülder, 2002), but in 1 study done on horses competing in an 80-km endurance ride, a reduction of plasma [alanine] after exercise to about 50% of the value before starting competition is described (Lucke and Hall, 1980a).

Only 2 other studies on the effect of a conditioning program on plasma [alanine] of horses were found (McKeever et al., 1986; Watermülder, 2002). McKeever et al. (1986) measured an increase of plasma [alanine] in resting horses after 14 d of conditioning with daily exercise at 1.6 m/s during no more than 20 min/d on a treadmill set at a slope of 12.5% incline. Compared with the present study, the work intensity applied by McKeever et al. (1986) on the horses was less, and duration of 1 exercise session as well as of the whole CDP was shorter. It may be that conditioning induces changes on resting concentrations of plasma [alanine] in horses at an early stage only. Thus, to demonstrate changes, it may be necessary to sample blood from the beginning of a study and at least at weekly intervals. Watermülder (2002) subjected Standardbreds to draft loaded training on a treadmill for 4 mo. Watermülder (2002) did not observe changes in the increase of the plasma [alanine] during exercise due to conditioning. Further studies are needed to disclose whether the plasma [alanine] reacts to conditioning.

In conclusion, the adaptations of the fitness variables measured and of plasma [FFA] and [alanine] to the conditioning programs used were not large. The lack of more distinct effects may have been because after an initial adaptation to the workload imposed, it was not increased further to induce larger adaptations. Therefore, it is strongly suggested that in all conditioning studies, workload is increased in periods of 2 to 3 wk to ensure measurable effects if there are any.

**LITERATURE CITED**


