EQUINE VETERINARY JOURNAL

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Equine vet. J. (2010) **42** (Suppl. 38) 171-178
doi: 10.1111/j.2042-3306.2010.00273.x

Morphological, haemato-biochemical and endocrine changes in young Standardbreds with ‘maladaptation’ to early training

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Keywords: horse; weight loss; sport stress; fatigue; hormones

Summary

Reasons for performing study: **The demands in the Standardbred trotters industry require young, still growing horses, to be trained well above light exercise level. During that period, the risk of occurrence of energy imbalance and maladaptation to training is high. In man, the lack of energy homeostasis is considered as the basic problem in the development of chronic fatigue.**

Objective: **To find objective biomarkers of early maladaptation to training in young racehorses under field conditions.**

Materials and methods: **Sixty-five 2-year-old Standardbreds were followed during their first 3 months of training in 5 different training centres. Monthly measurement of morphological variables (weight, height at withers, body condition score, body composition), basic haemato-biochemical variables and endocrine levels (testosterone, cortisol, thyroid hormones, leptin, IGF1, prolactin) were undertaken. Feeding levels and training programmes were also evaluated. At the end of the 3 month period, on the basis of an abnormal weight loss, 14 young horses were suspected of maladaptation to training (MT group). Morphological, haemato-biochemical, endocrine changes were compared between MT group (n = 14) and control group (C group, n = 40). Analysis of variance was calculated to study the effects of time and maladaptation to training.**

Results: **Compared to C group, MT group showed a significant higher weight loss in relation to a higher loss of fat mass and body condition score ($P<0.05$). MT group presented higher GGT and white cell counts and lower red cell counts ($P<0.05$). Finally, MT group showed significant lower levels of T4 ($P = 0.03$) than C group.**

Conclusion: **Some young horses presented signs of energy imbalance which were also associated with haematobiochemical and endocrine changes. Those markers might be useful for identification of maladaptation to training.**

Introduction

The athletic demands in the Standardbred trotters industry require young, still growing horses, to be trained well above light exercise level. The energy needs for such young horses consist not only of maintenance and growth but also of requirements for work. The risk of occurrence of energy imbalance during that period is high. In man, the lack of energy homeostasis is considered as the basic problem in the development of chronic fatigue potentially leading to overtraining (OT) syndrome. Moreover, chronic fatigue leads to downregulation of hypothalamic hormonal and sympathoadrenergic responses and catabolism (Steinacker *et al.* 2005).

In horses, overtraining is also considered as a cause of poor performance. As in human athletes, frequent signs of OT horses include behavioural disturbances, weight loss with correct feed intake, muscle soreness, increased occurrence of musculoskeletal accidents and higher sensitivity to infections. Overtraining-induced studies have been conducted over the past 15 years (Bruin *et al.* 1994. Tyler *et al.* 1996, 1998; Golland *et al.* 1999, 2003; Tyler-McGowan *et al.* 1999; Hamlin *et al.* 2002; McGowan *et al.* 2002; Wijnberg *et al.* 2008; de Graaf-Roelfsema *et al.* 2009). In these studies, the experimental difficulties reported are the identification of a clear loss of performance sustained for at least 2 weeks and the inclusion of a control group (Rivero *et al.* 2008). However, little is known about the occurrence of maladaptation to training under real training conditions. That field longitudinal study compared morphological, haematological and endocrine changes in a group of 14 young Standardbreds presenting signs of maladaptation to training to those observed in a group of 40 individuals serving as a control and the aim was to identify objective biomarkers of early maladaptation to training in young racehorses under field conditions.

Materials and methods

Horses

The population studied was initially composed of sixty-five 2-year-old Standardbreds, 28 males and 37 females. The mean age at the beginning of the experiment (mid-January 2009) was 632 ± 41 days (min: 556–max: 730 days). They were trained in 5 different training centres (respectively noted A, B, C, D and E) and were all prepared for qualifications beginning in June The qualification consists in racing 2000 m in a minimal mean speed of 12.2 m/s per kilometre.

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[Paper received for publication 15.01.10; Accepted 21.06.10]

Experimental design

On Days 0, 30, 60 and 90, respectively, noted T0, T30, T60 and T90, the young horses were measured in a standardised protocol. Sixty-five young horses were measured at T0. Eleven were excluded from the analysis because of incomplete data collection. Of these, 7 were withdrawn from training for lack of physical capacity and 4 for medical reasons. The analysis was based on 54 horses with complete evaluation.

Training evaluation

The training evaluation was based on answers to a standard questionnaire to trainers concerning the number of work sessions per week, the type, distance and intensity (speed) of each.

Feeding evaluation

A complete analysis of the ration was done in each stable. Qualitative information was evaluated on the basis of analysis of the roughage and concentrates. The nutritional data considered were the energy intake expressed in UFC (Anon 1990) and protein level expressed in MADC (Anon 1990).

Definition of maladaption to training

On the basis of the bodyweight changes during the 3 month period, 2 groups were considered: one group with a weight loss higher than 5 kg, considered to be a sign of maladaptation (noted MT group for group with maladaptation to training) and another group (noted C group for control group) with weight gain, stability or loss lower than 5 kg.

The mean age of MT group and C group was similar, respectively, 632 ± 47 days and 626 ± 34 days at T0. The MT group comprised 9 males and 5 females and C group 18 males and 22 females. The sex ratio was not significantly different in MT group and C group. Concerning the distribution of the MT group in the 5 stables, all the MT group horses were found in 3 stables: A, B, C. In the other 2 stables: D and E, all horses belong to the C group.

Although no objective measurements of behaviour and performance capacity were undertaken, trainers reported signs of irritability and unwillingness associated to stagnation or decrease in their ability to perform training sessions for all horses of the MT group.

Morphological measurements

Bodyweight and height at withers: Total bodyweight was measured with an electronic weight scale (Horse Weight)[1] with a precision of 500 g. The height at withers was measured manually always by the same operator.

Body composition: Rump fat thickness (RFT) was measured using ultrasound scan (Aloka)[2]. The site was determined by positioning the probe over the rump at 5 cm lateral from the midline at the centre of the pelvic bone (Westervelt *et al*. 1976). The region was scanned and the position of maximal fat thickness was used as the measured site. Percentage fat (% F) was estimated from the equation:

$$\% \mathrm{F} = 2.47 + 5.47 * \mathrm{RFT}\ (\text{in cm})$$

The CV for the measurement of rump fat thickness was 2%.

Body condition score: The body condition score was subjectively evaluated on a scale of 0–5, 0 being very emaciated and 5 extremely fat (Anon 1990). The evaluation used both the palpation of fat cover with hand and a visual appraisal of areas of fat deposits.

Haematology and biochemistry

Blood collection by jugular venepuncture was performed at rest, before the training, between 07.30 and 9.00 h. Blood was collected on EDTA tubes (haematology) and heparin tubes (biochemistry, endocrine parameters). The heparin tubes were immediately centrifuged and plasma frozen on dry ice and then stored at –30°C until analysis for biochemistry and endocrine variables.

Haematology

Basic resting haematology data: packed cell volume (PVC), haemoglobin concentration (Hb), red blood cells count (RBC), white blood cells count (WBC) were evaluated with automated analyser (Scil Vet abc)[3].

Biochemistry

Biochemistry analyses included: total serum protein (TP), creatine kinase (CK), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, γ-glutamyltransferase (GGT), bilirubin.

Endocrine evaluation

The endocrine evaluation was based on determination of plasma levels of:

Testosterone by radioimmunoassay using a commercially available kit (Spectria testosterone RIA)[4]. The testosterone within-assay and between-assay CVs were 5.5 and 4.8%, respectively.

Free thyroxine (T4) by radioimmunoassay using a commercially available kit (Immunotech FT4 RIA kit)[5]. The FT4 within-assay and between-assay CVs were 8.2 and 7.48%, respectively.

IGF1 by radioimmunoassay using a commercially available kit (IGF1 RIA-CT)[6]. The IGF1 within-assay and between-assay CVs were 4.6 and 6.2%, respectively.

Cortisol by radioimmunoassay using a commercially available kit (Diasorin CA-1529)[7], The cortisol within-assay and between assay CVs were 7.7 and 9.8%, respectively.

Leptin by radioimmunoassay using a commercially available kit (Linco multi-species leptin RIA kit)[8]. The leptin within-assay and between assay CVs were 3.6 and 8.7%, respectively.

Prolactin by enzyme immunoassay using a commercially available kit (Milenia Canine Prolactin MKVCP1)[9]. The prolactin within-assay and between assay CVs were 8.2 and 13.7%, respectively.

Statistical analysis

Mean ± s.e. of morphological, haemato-biochemical and endocrine variables were calculated and studied using statistical software (NCSS)[10]. Analysis of variance was calculated to assess effects of time and maladaptation to training. Duncan's *post hoc* tests were applied if a difference appeared and a nonparametric method used for semi-categorical variables. Level of significance of P<0.05 was considered throughout this study.

Results

Training evaluation

The training schedules in the different stables are described in Table 1. On the basis of training data, the number of exercises per week was the same in the 5 stables. The speed data were difficult to analyse as the precision of its assessment by trainers was found to be insufficient and because the corresponding work load is very 'track' dependent. Conversely, the distance covered on each work session or footing was found to be the most reliable piece of information given by trainers.

The global distance (high intensity sessions and low intensity sessions) covered on a week was significantly longer in stables A, B and C compared to stables D and E. In stables A, B and C, the mean global distance per week was 33.1 km per week whereas in stables D and E, the mean global distance was 21.3 km per week.

Feeding evaluation

As with their training schedule, stables A, B and C differed significantly from stables D and E in their nutritional programme. Concerning the energy intake, the mean amount of energy was significantly higher in stables A, B and C compared to stables D and E, with, respectively, 0.88 UFC/kg of dry matter (DM) compared to 0.8 UFC/kg MD. Conversely, the mean protein level was significantly lower in stables A, B and C (79 g MADC/kg DM) compared to stables D and E (89 g MADC/kg DM). In all stables, both energy and protein intakes were within or higher recommendations of INRA for young horse in training.

Morphological measurements

Table 2 shows mean ± s.e. of morphological variables in MT and C groups. Analysis of variance showed no group or time effect on body weight, even if a tendency for lighter weight in MT group appeared at T90. There was no difference of height at withers between MT and C group and no effect of time. Concerning body composition, the percentage fat was significantly affected by time and maladaptation, with MT group presenting a lower percentage of fat at T90 compared to C group (Fig 1). This result was confirmed by the evolution of body condition score also both influenced by time and fatigue. BCS was lower in MT group compared to C group at T30, T60 and T90 (P<0.05).

Haematology and biochemistry

Table 3 shows mean ± s.e. of haematological variables in MT and C groups. Analysis of variance showed no time effect on any haematological variables but a significant lower RBC and higher WBC (P<0.05) in MT group compared to C group (Fig 2).

Considering the whole population, total protein, AST, (GGT increased significantly during the period observed whereas ALP

TABLE 1: Description of training schedules in the five training centres

Stables	High intensity sessions	Low intensity sessions	Cumulated distance/week
A	2 sessions per week Warming-up: 3000 m at 4 m/s 2 × 2500 m at 11 m/s Recovery: 3000 m at 4 m/s	2 sessions per week Footing: 5000 m at 4 m/s	32 km
B	2 sessions per week Warming-up: 3000 m at 4 m/s 2 × 2800 m at 8.3 m/s uphill Recovery: 3000 m at 4 m/s	2 sessions per week Walking: 4500 m at 2 m/s	32.2 km
C	2 sessions per week Warming-up: 3000 m at 4 m/s 2 × 2800 m at 10 m/s Recovery: 1500 m at 4 m/s	2 sessions per week Footing: 7500 m at 4 m/s	35.2 km
D	3 sessions per week Warming-up: 3000 m at 4 m/s 2300 m at 10 m/s Recovery: 2000 m at 4 m/s		21.9 km
E	2 sessions per week Warming-up: 3000 m at 4 m/s 2100 m at 9 m/s Recovery: 1500 m at 4 m/s	1 sessions per week Footing: 7500 m at 4 m/s	20.70 km

TABLE 2: Morphological variables in groups MT (n = 14) and C (n = 40) between T0 and T90

	Group	T0	T30	T60	T90	MT effect	Time effect
Weight (kg)	MT	440 ± 9	429 ± 9	432 ± 10	426 ± 10^{b}	ns	ns
	C	430 ± 5	435 ± 5	439 ± 5	443 ± 5		
Height (cm)	MT	156 ± 1	156 ± 1	157 ± 1	157.5 ± 0.8	ns	0.08
	C	156 ± 0.7	157 ± 0.6	157 ± 0.6	158 ± 0.6		
BCS	MT	2.82 ± 0.08	2.64 ± 0.06*	2.66 ± 0.08*	2.39 ± 0.07*	0.000	0.000
	C	2.98 ± 0.08	2.91 ± 0.04	2.89 ± 0.04	2.85 ± 0.05		

Data are shown as mean ± s.e. *Differences between MT group and C group significant (P<0.05) BCS, body condition score; ns, not significant.

decreased. Creatinine increased between T0 and T60 and then decreased on T90. Considering the effect of maladaptation, the MT group presented significantly higher creatinine, (GGT) (Fig 3) and lower urea compared to C group. No difference in TP, CK, AST, ALP and bilirubin was observed between the 2 groups.

Endocrine variables

Table 4 shows mean ± s.e. of endocrine variables in MT and C groups. Considering the whole population, a significant effect of time was observed for all variables except the cortisol. Plasmatic levels of IGF1, testosterone, leptine and prolactin increased significantly, respectively, between T0 and T90, between T60 and T90, at T60 and between T0 and T30. On the contrary, T4 decreased significantly between T0 and T90. Considering the effect of maladaptation, MT group presented of lower levels of T4 ($P<0.05$) (Fig 4) and trends for lower IGF1 ($P = 0.06$) and leptin ($P = 0.06$) compared to C group. Testosterone, cortisol and prolactin were not different between the 2 groups.

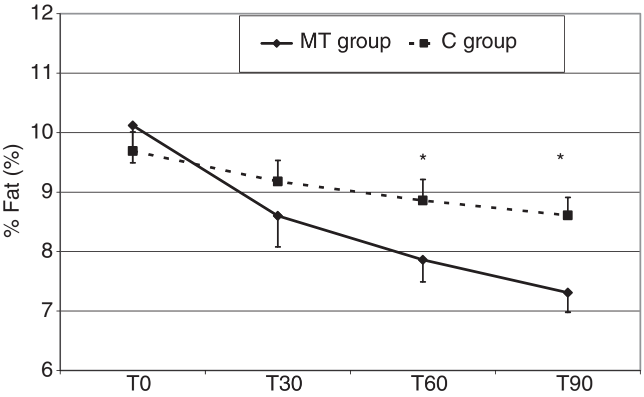

*Fig 1: Evolution of the % fat in MT group (n = 14) and C group (n = 40) between T0 and T90. Data are shown as mean ± s.e. * MT different from respective C value (P<0.05).*

Discussion

Limitations of the study

The most important limit of the study is the lack of performance evaluation and/or physiological evaluation at exercise. Modification of lactic metabolism (Hamlin *et al*. 2002), cardiac function (Hamlin *et al*. 2002; Kinnunen *et al*. 2006) or oxygen uptake (Tyler *et al*. 1996) at exercise have been reported in several studies of overtraining in horses. It would have been interesting to add that type of information to have multiple criterias to suspect maladaptation to training.

The 2-year-old racehorse population is particularly relevant to study maladaptation associated with training for several reasons. Their energy needs are very high due to the fact that they are still growing whilst following a physical training programme. As they have no history of training, their system is easily overwhelmed by the imposition of this physical workload. Finally, the programme imposed is generally standardised and cannot be individualised as the trainers do not yet know the capacity of each young horse. For all these reasons, 2-year-old horses are particularly easy to fatigue and can show signs of 'maladaptation' to training very quickly in comparison to mature horses.

Definition of maladapted group

An imbalance between training and recovery leads to a state of fatigue, which can be acute but also become chronic if the imbalance continues. Over-reaching (OR) is a state of accumulated fatigue, with or without decreased performance, which is followed by recovery and supercompensation within one or 2 weeks of rest or decreased training. Overtraining (OT) is the final state of fatigue with unexplained underperformance persisting despite 2 weeks of relative or complete rest (Rivero *et al*. 2008).

In the current study, the weight loss was considered as the criterium to qualify the horse of suspect of maladaptation to training. As no evaluation of physical performance was undertaken,

TABLE 3: Haematological and biochemical variables in groups MT (n = 14) and C (n = 40) between T0 and T90

	Group	T0	T30	T60	T90	MT effect	Time effect
RBC (10^6/mm^3)	MT	9.46 ± 0.2	9.28 ± 0.27	9.32 ± 0.21	9.56 ± 0.26	0.02	ns
	C	9.98 ± 0.19	9.55 ± 0.12	9.85 ± 0.18	9.73 ± 0.15		
Hg (g/dl)	MT	12.4 ± 0.29	12.8 ± 0.38	12.1 ± 0.29	12.9 ± 0.31	ns	ns
	C	12.7 ± 0.22	12.6 ± 0.14	12.2 ± 0.19	12.5 ± 0.18		
PCV (%)	MT	36.4 ± 0.82	36.5 ± 1.07	37.3 ± 0.81	38.7 ± 0.97	ns	ns
	C	37.5 ± 0.7	36.6 ± 0.47	38.4 ± 0.66	38.2 ± 0.57		
Urea (mmol/l)	MT	5.38 ± 0.16	5.02 ± 0.18	5 ± 0.18*	5.52 ± 0.34	0.01	ns
	C	5.87 ± 0.17	5.49 ± 0.17	5.61 ± 0.17	5.56 ± 0.19		
Creatinine (µmol/l)	MT	100 ± 4*	106 ± 3*	103 ± 2*	87 ± 4	0.000	0.000
	C	88 ± 2	98 ± 2	95 ± 2	82 ± 2		
TP (g/l)	MT	66.5 ± 1.3	66 ± 1.1	64.5 ± 1.25	67.1 ± 1.4	ns	0.01
	C	67 ± 0.5	65.5 ± 0.6	65.6 ± 0.5	68 ± 0.52		
CK (iu/l)	MT	228 ± 13	206 ± 10	212 ± 7	193 ± 10	ns	ns
	C	235 ± 14	229 ± 9	250 ± 18	330 ± 58		
AST (iu/l)	MT	319 ± 12	331 ± 9	381 ± 37	427 ± 71	ns	0.02
	C	334 ± 9	352 ± 10	395 ± 29	399 ± 32		
ALP (iu/l)	MT	246 ± 14	240 ± 8	220 ± 10	204 ± 8^b	ns	0.02
	C	238 ± 8	227 ± 6	222 ± 7	226 ± 6		
Bilirubin (μmol/l)	MT	21 ± 1.5*	24 ± 1.6	23 ± 1.6	24 ± 1.7	ns	ns
	C	26 ± 1	24 ± 0.9	25 ± 1.1	25 ± 1		

Data are shown as mean ± s.e. *Differences between MT group and C group significant (P<0.05). RBC, red blood cells count; Hg, haemoglobin concentration; PCV, packed cell volume; TP, total serum protein; CK, creatine kinase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ns, not significant.

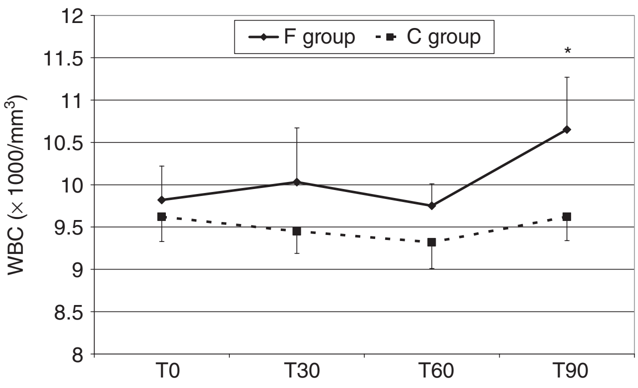

*Fig 2: Evolution of WBC in MT group (n = 14) and C group (n = 40) between T0 and T90. Data are shown as mean ± s.e. * MT different from respective C value (P<0.05).*

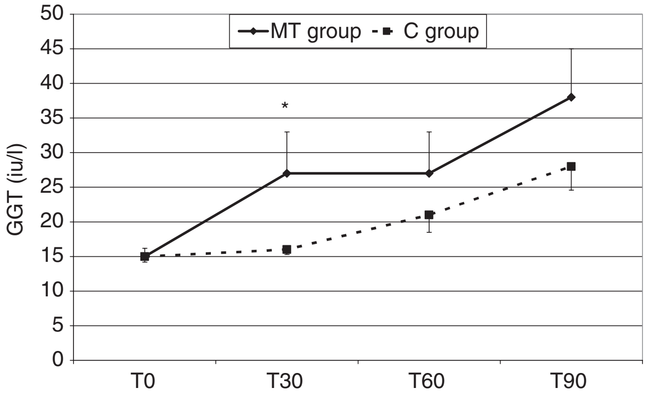

*Fig 3: Evolution of the (GGT activity in MT group (n = 14) and C group (n = 40) between T0 and T90. Data are shown as mean ± s.e. * MT different from respective C value (P<0.05).*

the terms of fatigue, OR or OT cannot be used here. The interests of such a variable are its easiness and reliability and also that it can be practically used by trainers.

Morphological changes associated with maladaptation to training

As in human athletes, weight loss is a critical sign in fatigued horses (Tyler *et al*. 1996; Hamlin *et al*. 2002). In the present study, the MT group presented an important weight loss (–12 kg) associated to a strong decrease in fat mass (–2.8%) and body condition score (–0.43) compared to C group which presented a weight gain (+13 kg) associated to a light loss of fat mass (–1%) and BCS (–0.1). Compared with other studies using mature horses in the OT model, these findings are highly abnormal as these young horses are still growing and therefore should not lose weight.

The energy imbalance was more likely due to an excess of energy expenditure rather than an insufficient energy supply. Indeed, the MT group was composed of horses belonging to stables having the higher level of physical activity and the higher level of energy intakes. Those intakes were within or higher than the recommended level for young horses. On the contrary, the 2 stables with a lighter training programme and lighter energy intakes (also within recommendations) did not show signs of maladaptation.

Haematological changes associated with maladaptation

We observed a significantly lower RBC in MT group compared to C group (P<0.05) with no time effect in any group. As the difference between the 2 groups was observed since T0, lower RBC could predispose to the development of signs of maladaptation to training in young horses. Interestingly, in Standardbred trotters, prolonged training was associated with an excessive increase in red cell mass and the phenomenon known as red cell hypervolaemia (Persson 1967; Pösö *et al*. 1993; Persson and Osterberg 1999). These horses have been suggested to be 'overtrained' due to excessive fast work training and racing and exhibit diminished exercise tolerance and signs of abnormal exhaustion during and after a race. However, several longitudinal and well controlled studies could not demonstrate a difference in red cell volume in horses with OT (Rivero *et al*. 2008). One common finding in horses undergoing high intensity training has been significant increase in resting red blood cell count. The increase in erythrocyte numbers is thought to result from a greater demand for oxygen carriage, which stimulates erythrocyte production.

Here, the absence of variation might highlight some of the problems in trying to interpret the resting haemogram. Its variability could be explained by the apprehension of the young horses unaccustomed to blood sampling at the beginning of the experiment or the increased excitability as they achieve higher levels of fitness.

Among haematological changes in the MT group, we noticed a leucocytosis at T90 compared to C group. A leucocytosis has been associated with overtraining in a recent study using a model of OT Wistar rats (Hohl *et al*. 2009) and also in OT humans (Margonis *et al*. 2007). In both man (Yaegaki *et al*. 2008) and horses (Raidal *et al*. 2001), it has been demonstrated that neutrophil function was affected by OT and that the detrimental effects observed in peripheral blood neutrophil could indicate an impaired nonspecific immunity.

As regards biochemical changes, the increase in (GGT was much higher in MT group than in C group. This finding is in accordance with the field observation of Snow *et al*. (1987). They also observed a general rise of (GGT activity as the intensity of training increased. The authors reported that excessive increases in plasma (GGT activities may occur in those animals where the training stress was too severe and concluded that the monitoring of plasma GGT activity may provide a sensitive indicator's of the horse's response to training. Although this finding has been associated with poor health and overtraining, it has not been verified in controlled OT studies (Rivero *et al*. 2008).

Endocrine changes associated with maladaptation to training

Even if there is no specific biological marker of fatigue induced by training, it seems clear that endocrine levels can be affected by fatigue and OT. A hypothalamic-pituitary dysfunction is suggested as the underlying cause of OT (de Graaf-Roelfsema *et al*. 2007). In the current study, plasma levels of T4 were lower in MT group than in C group, and trends for lower IGF1 and leptin were also noticed. Testosterone, cortisol and prolactin were not different between the 2 groups.

T4

We observed a decrease of T4 level in both groups between T0 and T90 and significant lower values in MT group compared to C

TABLE 4: Endocrine variables in groups MT (n = 14) and C (n = 40) between T0 and T90

	Groups	T0	T30	T60	T90	MT effect	Time effect
IGF1 (ng/ml)	MT	266 ± 18	342 ± 20	330 ± 15	398 ± 27	0.06	0.000
	C	276 ± 9	368 ± 14	363 ± 9	412 ± 12		
Leptin (ng/ml)	MT	1.58 ± 0.19	1.28 ± 0.11	2.27 ± 0.2	1.5 ± 0.23	0.06	0.000
	C	1.95 ± 0.2	1.64 ± 0.12	2.38 ± 0.2	1.82 ± 0.16		
Testosterone (nmol/l)	MT	1.6 ± 0.3	2 ± 0.3	1.4 ± 0.5	2.3 ± 0.5	ns	0.05
	C	1.6 ± 0.3	2.3 ± 0.4	1.6 ± 0.3	3.2 ± 0.5		
Cortisol (nmol/l)	MT	82 ± 5	79 ± 6	73 ± 4	89 ± 5	ns	ns
	C	97 ± 5	81 ± 5	81 ± 5	92 ± 5		
Prolactin (ng/ml)	MT	4.5 ± 3	23 ± 8	15 ± 5	11 ± 5	ns	0.000
	C	5 ± 2	13 ± 3	18 ± 3	15 ± 3		

Data are shown as mean ± s.e. *Differences between MT group and C group significant ($P<0.05$). ns, not significant.

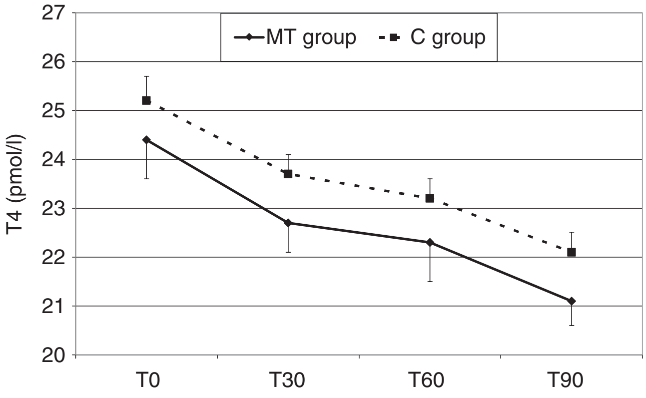

Fig 4: Evolution of T4 in the MT group (n = 14) and C group (n = 40) between T0 and T90. Data are shown as mean ± s.e.

group. Our values of T4 are in accordance reported references ranges for normal horses (Chen and Riley 1981). In horses, several factors can influence the concentration of thyroid hormones such as diurnal variation (Duckett *et al*. 1989), seasonal variation (Irvine 1982; Buff *et al*. 2007), age (Malinowski *et al*. 1996), energy availability (Messer *et al*. 1995; Powell *et al*. 2000), training (Irvine 1967, 1982), exercise (Graves *et al*. 2006) or medication (Sojka *et al*. 1993). The significant decrease of T4 over the 90 day period might be due to a combination a several factors including seasonal change with warmer temperatures at T90, ageing, training and also energetic imbalance, these 4 factors leading individually to a decrease of T4. The standardisation of timing of sampling and delay from the latest training session allowed control of diurnal variations and hormonal changes due to exercise. However the lack of a control group without training makes discrimination impossible between training and ageing effects.

Interestingly, in human athletes, Hesse *et al*. (1989) found that younger marathon runner and best performers had significantly higher T4 concentrations whereas older sportsmen and worst performers reacted to the stressful stimulus with a significant decrease of T4. This response was interpreted as an exhaustion reaction, i.e. reduced adaptation responses of thyroid hormone metabolism.

IGF1

The main physiological role of IGF1 is to increase the rate of protein synthetis. In a review, de Graaf-Roelfsema *et al*. (2007) reported that in man, the effect of training and overtraining on growth hormone (GH) axis was contradictory. Decrease in IGF1 levels or GH levels were sometimes observed at rest or after exercise even if those changes could not be confirmed in other studies. Other makers of GH axis, like IGFBP-3, have been proposed as more sensitive markers of overtraining in man (Elloumi *et al*. 2005). In a recent study, de Graaf-Roelfsema *et al*. (2009) demonstrated that the resting pulsatile growth hormone secretion of overtrained horses was modified but their basal IFG1 was not different from the control group.

Leptin

Chronic exercise reduces body fat mass, which may result in a decreased blood leptin concentration. Leptin level has been related to the intensity of physical training and possibly to overtraining in a study involving young female swimmers of various training levels (Zietz *et al*. 2009). However, in overtrained human athletes, plasma leptin was not significantly modified (Ishigaki *et al*. 2005) and its modifications not correlated to other changes associated with overtraining: loss of fat mass, decrease in testosterone levels and increase in cortisol. The influence of a decrease in leptin level could be an inhibition of hypothalamic function. Leptin (but also insulin and interleukin 6) could contribute to the metabolic error signal to the hypothalamus which results in a decrease in hypothalamic released hormones (like TSH) and sympathoadrenergic stimulation (Steinacker *et al*. 2004, 2005).

Cortisol

In the current study, basal cortisol concentration was not different between MT and C group and no significant change over time was observed. Bruin *et al*. (1994) found no change in basal cortisol during a short-term overtraining. However, Hamlin *et al*. (2002) reported a decrease in basal cortisol level in overtrained Standardbreds.

The effects of training and OT on plasma cortisol and concentrations at rest and after standardised exercise tests and the cortisol responses to adrenocorticotropin (ACTH) administration were also investigated in Standardbred horses (Golland *et al*. 1999). Peak cortisol concentrations after exercise decreased significantly in the OT group as well as mean cortisol concentrations over a 120 min period after exercise. However, mean and total cortisol concentrations in resting horses were not significantly different in OT group. Peak cortisol concentrations after adrenocorticotropin (ACTH) administration were not significantly different in the OT group. The authors concluded that dysfunction of the hypothalamo-pituitary-adrenocortical axis occurs in OT horses, but this adaptation is not associated with a change in the adrenocortical responsiveness to ACTH. Because of circadian rhythm and the strong effect of physical or psychological stress, plasma concentration of total cortisol may not accurately reflect changes in the hypothalamic-pituitary-adrenal axis (de Graaf-Roelfsema *et al*. 2007).

Conclusion

The aim of this field study was to compare morphological, haematological and endocrine changes in a group of 14 young Standardbreds presenting signs of maladaptation to training to those observed in a group of 40 individuals serving as a control. Considering basic information such as the weight evolution, a 'maladaptation' to training of these growing horses was suspected in 25% of the studied population. The energy imbalance, confirmed by the importance of loss of fat mass, was associated with changes in some haematological, biochemical and endocrine variables. Some of those have been associated to overreaching or overtraining in other studies. The analysis of the training schedule and nutrition programme showed that the energy imbalance was, in that study, more likely due to an excess of energy expenditure rather than an insufficient energy supply. The young horses are particularly concerned by the risk of energy maladjustment as their energy needs are very high due to the end of growing period associated to a more and more demanding level of physical activity. As both nutrition and training influence this delicate equilibrium, precise knowledge of their interaction is needed. Follow-up of the young equine athletes could then be proposed to trainers to optimise the nutritional and training programmes.

Acknowledgements

The authors wish to express their gratitude for funds received from the French National Stud.

Conflicts of interest

None declared.

Manufacturers' addresses

[1]Horse weight, Wales, UK.
[2]Aloka, Tokyo, Japan.
[3]Scil Vet abc, Scil animal care company, Gurnee, USA.
[4]Spectria Testosterone RIA, Orion Diagnostica, Espoo, Finland.
[5]FT4 RIA KIT, Immunotech, Prague, Czech Rep.
[6]IGF-1 RIA CT, Mediagnost, Reutlingen, Germany.
[7]CA 1529 E, Diasorin, Stillwater, USA.
[8]Multi-species leptin RIA kit, Linco research, St Charles, USA.
[9]Milenia Canine Prolactin, Milenia biotec, Giessen, Germany.
[10]NCSS, Kaysville, USA.

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