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Biochemical Aspects of Overtraining in Endurance Sports

A Review

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Abstract

Top-level performances in endurance sports require several years of hard training loads. A major objective of this endurance training is to reach the most elevated metabolic adaptations the athlete will be able to support. As a consequence, overtraining is a recurrent problem that highly-trained athletes may experience during their career. Many studies have revealed that overtraining could be highlighted by various biochemical markers but a principal discrepancy in the diagnosis of overtraining stems from the fact that none of these markers may be considered as universal. In endurance sports, the metabolic aspects of training fatigue appear to be the most relevant parameters that may characterise overtraining when recovery is not sufficient, or when dietary habits do not allow an optimal replenishment of substrate stores. From the skeletal muscle functions to the overall energetic substrate availability during exercise, six metabolic schemes have been studied in relation to overtraining, each one related to a central parameter, i.e. carbohydrates, branched-chain amino acids, glutamine, polyunsaturated fatty acids, leptin, and proteins.

We summarise the current knowledge on these metabolic hypotheses regarding the occurrence of overtraining in endurance sports.

Fatigue has been defined as an inability to maintain a given exercise intensity[1] and may be considered as an alarm signal from the organism indicating a stress situation diminishing its initial capacities. On the other hand, fatigue induction through training exercises is the first rule of the training adaptation process to improve athlete capacities by stimulating organism functions. The balance between stress and recovery factors defines the quality of the training programme. However, the stress limits and minimal recovery periods an athlete must use to optimise his/her training programme are not known and must be individualised. To date, to optimise training, it is very tempting to reduce the recovery periods and increase the training loads as long as fatigue seems bearable. Overstepping this maximal ability to train may result in a fatigue accumulation, and possibly overtraining. One consequence is a greater susceptibility to various pathologies, such as asthenia,[2] upper respiratory tract infections (URTIs)[3] and viral or bacterial infections,[4] which may be attributed to an impairment of the body defences.[5] Another consequence is that recovery from overtraining may take several weeks or months without physical activity before returning to training again.[6]

It has been estimated that 70% of high-level endurance athletes have experienced (or will experience) overtraining during their career.[7] However, the exact aetiology of overtraining is not fully understood[8] and there is no universal tool to predict its occurrence before it is clinically diagnosed. The only available diagnostic tool is a recurrent performance decrement while maintaining or increasing training load.[9] This late diagnosis may result in the loss of several months in the training programme due to a long-lasting recovery period. Therefore, diagnostic tools are needed to recognise new cases of overtraining and, if possible, to prevent its occurrence. For endurance sports, there is a growing body of evidence that overtraining stems from dysfunctions in carbohydrate, lipid, and/or protein metabolism.

1. Metabolic Aspects of Overtraining

In endurance sports, a heavy training load consisting of repetitive long-duration exercises at specific intensities is necessary to enhance given metabolic pathways for energetic supply to skeletal muscles. The major metabolic adaptations to endurance training load have been situated within the skeletal muscle cell,[10] liver,[11] and kidney.[12] Indeed, these locations are potentially implied in metabolic aspects of the overtraining process. Another consequence is that the study of overtraining requires taking into account parameters from different biological tissues and/or to combine different analytical approaches, i.e. biochemical, physiological, endocrine, neuronal, myologic; each one being potentially involved in the understanding of the metabolic aspects of overtraining. In the past two decades, many studies have been conducted to investigate given biochemical parameters implied in the occurrence of overtraining or to diagnose it. There is a growing body of evidence that only a limited number of metabolic schemes may highlight overtraining occurrence. To date, six metabolic schemes seem to provide pertinent information about overtraining occurrence. In this review, we propose to summarise the current knowledge on these metabolic schemes, each one centred around a key parameter, i.e. carbohydrates,[13] branched-chain amino acids (BCAA),[14] glutamine,[15] polyunsaturated fatty acids (PUFAs),[16] leptin,[17] and proteins.[6] As a starting point in this review, the hypothesis that mechanical and/or chemical stress on myocytes may favour or induce overtraining is presented.

1.1 The Skeletal Muscle Structure Hypothesis

During intense and/or eccentric exercises, myocyte alterations may be induced, either by mechanical (disruption of cellular architectural pro-

teins) or metabolic stress (chemical aggressions on subcellular contents). During oxido-reduction processes, highly reactive oxygen species (ROS) are continuously produced since 1 to 3% of oxygen is incompletely reduced.[18] Successive reactions may occur from ROS, producing superoxide anions (O_2–) that may induce peroxidation of the phospholipids located within skeletal muscle cell membranes.[19] Hydroperoxide (H_2O_2) may also be produced and this generates hydroxyl radicals (OH) in the presence of Fe^{2+}, which are highly reactive and attack other families of biomolecules, namely proteins, DNA and lipids. Lipidic radicals may also be produced from hydroxyl radicals by subtracting hydrogen to PUFAs, which leads to the formation of lipoperoxyl (LOO) and alkoxyl (LO) radicals, and aldehydes (malondialdehyde) as by-products. These have been found to alter skeletal muscle cell membrane functions.[20] Peroxidation of the lipids located in cellular membranes have been observed during intense exercise by measuring plasma malondialdehyde concentration.[21] It has also been postulated that superoxide anion production (O_2–) might induce oxidation of the catecholamines implied in the mobilisation of substrates used by skeletal muscles during endurance exercise.[22]

During exercise, oxygen consumption may increase up to 40-fold. As a consequence, ROS production is also greatly increased.[21] A defence system exists, which includes enzymatic and non-enzymatic (vitamin) actions to reduce ROS aggressions within skeletal muscle cells. This defence system may be enhanced by endurance training. An imbalance between ROS actions and antioxidative defence capacities of skeletal muscle cells has been suggested to be a potential factor facilitating overtraining occurrence.[23] However, it has never been demonstrated that a long-term peroxidation of skeletal muscle cell biomolecules (phospholipids and contractile proteins) could sufficiently alter the cellular functions to induce overtraining.[19,20,24] Nevertheless, it is well known that ROS actions increase membranes' permeability, releasing several cellular biomolecules that may be measured within blood, namely creatine phosphokinase (CPK), myoglobin, skeletal troponine I (sTi) and 3-methylhistidine. Blood release of 3-methylhistidine results from contractile protein degradation and its concentration may remain elevated up to 72 hours after an exhaustive endurance exercise.[25] The CPK enzyme activity depends on the cytosolic biochemical equilibrium for the coupling between metabolic (myoglobin) and contractile (sTi) proteins. It has been proposed to measure CPK blood concentrations during and after intense endurance exercises to study athletes' recovery capacities.[26,27] However, since membrane permeability usually remains elevated 48 to 96 hours after exercise, the CPK release within the blood stream will necessarily follow the membrane restructuring kinetic.[9,28] Moreover, this enzyme diffuses within the blood stream whether skeletal muscle cell damage is reversed in a few hours or not, and its presence is constant in most cells of the organism for adenosine triphosphate (ATP) resynthetic processes. Indeed, whatever the importance of the skeletal muscle cell damage, blood CPK may be found to be elevated after intense exercises.[29,30] As a consequence, its utilisation for highlighting overtraining occurrence during heavy training periods would be very difficult to appreciate. Myoglobin is a free cytosolic protein responsible of O_2 transport into mitochondria. It has been shown that this protein diffuses easily within the blood stream while cellular membrane permeability increases.[31] Oxidative-type fibres are the most exposed to ROS peroxidations, leading to abundant release of myoglobin out of the skeletal muscle cell area. The study of its blood concentration kinetic following intense endurance exercises appears to be useful in estimating the chemical stress level in skeletal muscle cells and to give information about the fibre which is the most damaged.[31] However, it has never been demonstrated that the muscular proteins found in high concentrations within blood could be sensitive parameters for discriminating reversible training fatigue from overtraining.[32] Peroxidation processes do not appear to induce overtraining since skeletal muscle cell

destructuring is one of the most important sources of feelings of stiffness for athletes, as well as eccentric exercises that disrupt cellular components.[19] Muscular pains appear as alarm signals and inhibit athletes' capacities to realise other intense exercises.[33] Indeed, it seems unlikely that overtraining might appear as a consequence of successive alterations in the skeletal muscle system,[34] but rather that skeletal muscle cell damage may participate in the overtraining process.

1.2 The Carbohydrate Hypothesis

During endurance exercise, fatigue may induce a slight transient hypoglycaemia, which is due to hepatic and/or muscular glycogen store depletion, and/or to a failure in glycogenolytic metabolic flux. Following several intense and long-lasting endurance training sessions, glycogen depletion may become chronic if carbohydrate ingestion is inappropriate,[35] leading to its slower and delayed repletion.[36] It has been found that exercise hypoglycaemia could have higher severity in overtrained athletes[8,13] and that the lactacidaemia increase could be lower,[37-39] suggesting a poor participation of glycolysis to skeletal muscle metabolism. As a consequence of this poor glycolysis, purine nucleotide metabolism would be prolonged over the hydrolysis of ATP and adenosine diphosphate (ADP), which may produce higher amounts of inosine monophosphate (IMP) and NH_{4+}.[11] This process is known to release by-products such as hypoxanthine and xanthine oxidase which are toxic if found in high concentrations within the muscle cells. However, although overtrained athletes present higher glycogen depletion in response to long-term endurance exercises, glycogen store replenishment between exercises is generally found to be optimum.[39,40] Indeed, rather than being responsible for the occurrence of overtraining in endurance-trained athletes, these repeated glycogen depletions might induce subtle changes within the metabolic pathways that contribute to the skeletal muscle energy supply.[13,41] It has been suggested that long-term glycogen depletion would lead to an increased BCAA oxidation, which is much more likely to be responsible for a central fatigue process.[13]

1.3 The Branched-Chain Amino Acid Hypothesis

During endurance exercise, BCAA (leucine, isoleucine, valine) may be widely captured by skeletal muscles (but not the liver), to be oxidised for ATP resynthesis.[42] Along with BCAA, plasma free fatty acids (FFA) may also be oxidised in higher amounts by skeletal muscles as glycogen stores are depleted.[43,44] FFA are not water soluble and thus necessitate albumin binding to be transported into the blood stream. However, there is competition between tryptophan and FFA binding to albumin for blood transport. Indeed, an increase in FFA transport to skeletal muscles induces a higher utilisation of albumin transport capacities. In turn, this albumin utilisation leads to the blood release of free tryptophan.[45] BCAA and aromatic amino acids utilise the same carrier in the haematoencephalic barrier. Thus, a consequence of the increase in free tryptophan concentration (while that of BCAA decreases), is the facilitated free tryptophan entry into the cortical area.[14] Cerebral tryptophan is then converted to serotonin, in specific cortical areas. This serotonin may have several functions: (i) sleep induction; (ii) motoneuron excitability and inhibition of post-synaptic reflexes (notably during exercise);[43] and (iii) endocrine functions inhibiting the release of hypothalamic hormones, which may impair various endocrine regulations within the body.[46] Such phenomena have been observed in overtrained athletes.[14] Therefore, a decrease in the free tryptophan to BCAA blood concentration ratio (free tryptophan/BCAA ratio) has been proposed as a diagnostic tool for detecting overtraining in endurance athletes.[47]

However, ingestion of BCAA during or after endurance exercise has not been shown to restore the free tryptophan/BCAA ratio and did not significantly change the loss of performance level due to metabolic fatigue induced by glycogen store depletion.[44,48] In fact, ingestion of BCAA increased

amino acid carbon skeleton drainage through the tricarboxylic acid cycle to form acetyl-CoA. This cycle leads to ATP production from amino acid carbon skeletons, but ammonium ions will also be produced in large amounts, rapidly becoming toxic for the muscle cell. In skeletal muscle cells, BCAA represent the first amino group donor to 2-oxoglutarate for glutamate formation. Glutamate leads to glutamine synthesis via glutamine synthase. This is the main biological ammonium vector, a compound highly toxic when free.[42] Thus, the ingestion of BCAA did not appear useful in decreasing the high free tryptophan/BCAA ratio observed in overtrained athletes because of the potential induction of other metabolic stress.[48] Furthermore, the relationship between the higher depletion of glycogen stores observed in overtrained athletes and the central fatigue potentially induced by the free tryptophan/BCAA ratio increase has not been described. It has not been demonstrated whether the free tryptophan/BCAA ratio remains significantly elevated between intense endurance training sessions, while glycogen store replenishment is optimum.[49] Therefore, the continuous serotonin secretion that may appear during heavy training load does not seem to be a sufficient central fatigue inductor to lead to overtraining in endurance athletes. Regarding other metabolic aspects of overtraining, the link between BCAA oxidation during training exercises and serotonin secretion is one of the factors that may increase the susceptibility of athletes when experiencing the overtraining syndrome, i.e. along with other central and/or peripheral fatigue inductors.

1.4 The Glutamine Hypothesis

Glutamine is one of the most abundant amino acids within the human body. It is metabolised by immune cells, such as lymphocytes and macrophages.[50] Their proliferation depends on glutaminolysis,[51] suggesting that a decrease in blood glutamine concentration may at least be partially responsible for immune function deficiency or impairment. A portion of the glutamine found within the body enters the gut to be metabolised. Skeletal muscle appears as the most important glutamine producer, which is further released within the blood stream. Indeed, during an intense endurance exercise, the blood glutamine concentration constitutes a metabolic link between active skeletal muscles and immune system capacities of reaction.[52] Under catabolic stresses, such as infections, surgical interventions, trauma, burns and acidosis, glutamine homeostasis is placed under stress. Glutamine stores, notably within skeletal muscles, may be widely depleted. With respect to glutamine metabolism, exercise may also be considered as a catabolic situation.[15]

Intense endurance exercises induce a biphasic response in glutamine blood concentration. Firstly, it augments during exercise and, secondly, it falls significantly during rest periods, for several hours before again reaching the basal concentration. Indeed, it may be argued that insufficient rest periods between intense training sessions may limit glutamine release from skeletal muscles and, therefore, the immune system may become much more stressed.[52] Gut function may also be unsettled by this lower glutamine disposition, leading to higher risks of bacterial and viral translocations within organisms. Important glutamine depletions have been reported in overtrained athletes experiencing URTI.[4] A significant relationship between URTI frequency and the prolonged decrease in glutamine concentrations has also been reported in overtrained endurance athletes.[53,54] Indeed, the persistence of this situation would contribute to the occurrence of overtraining, these infections playing the role of immune stressors while a metabolic stress fatigue is already experienced by the athlete. However, decreases in glutamine concentrations have not been systematically observed in overtrained athletes and URTIs may appear with the same frequency in well-trained and overtrained athletes.[55] Moreover, immunosuppression has been observed in overtrained athletes, but without decreases in glutamine concentrations and in the absence of URTIs.[2,3,56]

1.5 The Polyunsaturated Fatty Acid Hypothesis

Immunosuppression seems to be recurrent in overtrained athletes. An alternative metabolic scheme has been proposed, starting with inhibition of lymphocyte proliferation due to PUFAs. It has been suggested that PUFAs may cause this inhibition with a higher chronicity than saturated fatty acids.[16] Exercise metabolic stress increases the blood concentration of fatty acids, notably during and after intense endurance exercises since glycogen stores are depleted. Lymph nodes are associated with adipose tissue. Thus, during fatty acid mobilisation from adipocytes, lymph nodule cells may be exposed to high PUFA concentrations. Depending on the fatty acids contained in adipose triglycerides, their high mobilisation during endurance exercise would lead to a lymphocyte proliferation inhibition in lymph nodules, along with an increased PUFA flux. An elevated sensitivity of adipocytes to lipolytic hormones, and a triglyceride composition in fatty acids shifting towards PUFAs may also be implied in the immunosuppression observed in overtrained athletes.[57] However, this immunosuppression has not been demonstrated in overtrained athletes, which should take into account the turnover and differentiation of fatty acids synthesised for triglyceride stores replenishment between intense endurance training sessions.

1.6 The Leptin Hypothesis

Leptin, the product of the ob-gene, is specifically released by adipocytes and reflects the body fat content. In addition to its metabolic functions, i.e. a putative satiety signal in humans, it seems to affect the feedback mechanisms of the hypothalamic-pituitary-gonadal-axis. Leptin secretion is complexly regulated in humans. Insulin has been shown to stimulate leptin secretion, whereas *in vitro* data suggest that catecholamines and FFAs inhibit leptin secretion. It has been shown that short-term exhaustive exercise has no immediate or delayed effect on circulating leptin concentration.[58] On the other hand, several studies showed that endurance exercise sessions decrease the plasma leptin concentration after 48 hours, in association with a preceding decrease in insulin.[59] Nevertheless, its long-term effects on metabolic adaptations to training remain controversial. Globally, serum leptin levels decrease in highly-trained endurance athletes in comparison with nonsporting individuals. Serum leptin levels in top-level athletes parallel the changes in body fat content and are not an independent predictor of endurance training level. It seems that plasma leptin is not sensitive to an increase in training volume for trained individuals. In fact, training level induced by resistance and/or endurance exercises did not influence leptin production when considering variations in body composition.[60]

Leptin, as well as inhibin B, colecalciferol (vitamin D_3), and possibly activin and resistin, are now considered as indicators of tissue overload in highly-trained endurance athletes. It has recently been suggested that metabolic functions of these hormones may become potent biochemical markers of overtraining occurrence in endurance sports.[17] Correlations have been found between variations in these neuroendocrine axis parameters and performance in fatigued athletes after 3 weeks of excessive training. The underlying mechanisms might help us to understand how overloaded peripheral organs and tissues 'tell' the brain of their fatigue. However, there are no studies on leptin levels regarding overtraining occurrence.[60] Data from recent studies strongly suggest plasma leptin is not sensitive to an increase in training volume and that this hormone may not be indicative of changes in fat mass with an increase in training volume in female athletes. These data suggest that leptin may not be useful in monitoring relative training stress in athletes.[61,62] Furthermore, the authors found no evidence of alterations in leptin levels in patients with chronic fatigue syndrome,[63] a fatigue syndrome thought to be close to overtraining in its biological effects on the human body.[34,64] Therefore, to date, the monitoring of changes in leptin levels along training duration

and intensity does not appear to be a useful tool in diagnosing or preventing overtraining. Nevertheless, in conjunction with other stress hormones, leptin may interfere in overloaded tissues in inducing a preventive resistance to further metabolic stress. This mechanism might be responsible for a down-regulation of the carbohydrate–lipid metabolism during exercise, facilitating the occurrence of overtraining.[17]

2. The Protein Metabolism Hypothesis

As a general rule, overtraining has not been associated with important variations in blood protein content.[5,53,65] However, an intense endurance exercise strongly augments metabolic processes within skeletal muscles, liver, and kidney, which may be associated with tissue inflammation.[66] This inflammation induces a short-term response of hepatic proteins, i.e. fibrinogen, haptoglobin, C-reactive protein, α_1-acid glycoprotein, and α_1-antitrypsin, through their antiproteolytic functions.[67] Strenuous endurance training may cause three levels of inflammation: (i) firstly, this may be observed through a modest increase in α_1-antitrypsin concentration, along with heavy training loads, but without change in ferritin concentration; (ii) the subsequent level indicates a severe affection, characterised by higher increases in α_1-antitrypsin and ferritin concentrations; (iii) the later appears during particularly hard and heavy endurance training loads, which may cause important iron losses followed by long-term decreases in haptoglobin blood contents, and a rise in ferritin and α_1-antitrypsin concentrations. These events may be observed up to 24 to 48 hours after training.[68]

Continuation of this inflammatory situation may cause a substantial depletion in functional iron body stores. On the other hand, such depletion may occur after anaemia due to a mechanically induced haemolysis (trauma, repeated shocks, haematoma) and/or induced chemically by ROS action. In addition to the metabolic stress induced by exercise, peroxidation processes alter the cellular membrane functions of erythrocytes, facilitating their dehydration. A possible consequence is a disruption of erythrocyte ionic homeostasis that may limit their entry into the micro-circulation. This mechanism slightly augments hypoxia within active skeletal muscles, which may increase ROS action on the membrane phospholipids of erythrocytes; in turn, this may lead to erythrocyte destruction and, potentially, to exercise anaemia.[69] However, it has been shown that erythrocyte destruction during endurance exercise is not important enough to become deleterious for athletes. To the contrary, this slight exercise anaemia might be an interesting way for *de novo* synthesis of erythrocytes, leading to the blood release of young and potentially more efficient erythrocytes. Nevertheless, successive haematuria during endurance exercises may cause a rapid and significant decrease in blood haptoglobin, haemoglobin, haemopexin, and ferritin concentrations.[70] Long-term and repeated depletions of these protein stores along intensive endurance training may weaken muscle and liver defences against inflammatory processes. Skeletal muscle inflammation may also be associated with the catabolism of contractile proteins and with myofibril degeneration, in addition to the normal exercise protein turnover.[71] Nevertheless, exercise anaemia has not been clearly associated with the occurrence of overtraining since overtrained athletes do not present with important and long-term depletions in haptoglobin, haemoglobin, haemopexin, and ferritin concentrations.[30,65] Finally, these processes do not appear to cause overtraining in endurance athletes, but rather increase the metabolic stress and/or muscular and hepatic tissue inflammation that may lead to a chronic fatigue accumulation.[57,69]

Another index of the protein metabolism status is the ratio between free testosterone and cortisol concentrations (T/C ratio). This ratio has been proposed as a marker of the anabolic–catabolic status of the athlete, i.e. a global appreciation of protein turnover.[6,29,72] A drop in this ratio of 30% below the basal values of the athlete, i.e. before exercise or training, and/or values $<0.35 \times 10^{-3}$ may be in-

dicative of a too intensive training load[23] and may reveal overtraining in sprint and strength sports.[72] However, several studies on high-intensity resistance exercise overtraining failed to obtain significant changes in the T/C ratio, notably when overtraining occurred after several weeks of intensified training load.[5,6,9,53] Furthermore, athletes' ability to train in endurance sports is not primarily dependent on protein metabolism, but rather on energy metabolism, i.e. that which involves mainly carbohydrates and lipids. Therefore, a biological index such as the T/C ratio may not be sufficiently discriminatory to diagnose overtraining if it is not used in conjunction with biological markers of the energy status of the athlete.

3. Validity of Clinical Analyses to Diagnose Overtraining

The main difficulty in the diagnosis of overtraining is the need for repeated analysis of the blood prior to and after exercise. Moreover, the biochemical markers of overtraining may vary with respect to the characteristics of the sport practised and the nature of the training load. Many factors may also interfere with the biochemical aspects of overtraining, i.e. psychological, social, or cultural.[5] Furthermore, until recently, only a few studies have taken into account the phenomenon of exercise-induced changes in plasma volume. As recently reviewed,[73] cold exposure, psychological stress, nutrition, hydration, and the duration and intensity of exercise, may markedly change exercise haemoconcentration. Thus, the comparison of data obtained from different studies on overtraining remains globally impossible. This methodological point will be of major importance in the future since biochemical parameters of overtraining appear to be much more highlighted by exercise than rest analyses.[8,15,39,41,47,65,66,74] Therefore, the diagnosis of overtraining remains as a state of the art; possible in some cases[8] but unpredictable in many others.[34] To date, a list of potential markers of overtraining in endurance sports may be proposed (table I). However, there is still no single biochemical marker to propose as a signal of overtraining, i.e. to assess the limit between reversible training fatigue and overtraining.

3.1 Summary of Biochemical Markers of Overtraining

From the skeletal muscle function to the overall energy substrate availability during exercise, six metabolite pools have been studied in relation to overtraining, each one linked to a central parameter, i.e. carbohydrate, BCCA, glutamine, PUFA, leptin, and protein (table I). It is clear that in endurance sports overtraining may appear after several months of hard training.[34] Indeed, to diagnose, or rather to prevent overtraining, clinical analyses should be addressed throughout the training programme. Ideally, to ensure the best diagnosis of overtraining, every metabolite which has physiologically been linked to overtraining should be analysed (table I). However, in practice, this is impossible as it would require testing: (i) at rest, for comparison with the normal physiological range of each metabolite; (ii) after an exercise specific to the sport practice, in order to evaluate the responses of the athlete to normal training stimuli; and (iii) 24, 48, and 72 hours after this exercise in order to evaluate recovery capacities of the athlete and this adaptation to the training load. Our knowledge now extends, at the cellular level of skeletal muscle, to overload of oxidative stress and/or mechanical aggressions being abnormally elevated, and can be monitored by studying the plasma kinetics of CPK, malondialdehyde, tocopherol (vitamin E), ascorbic acid (vitamin C), retinol (vitamin A), myoglobin, 3-methylhistidine, and sTi. Alterations of the energy metabolism may also be highlighted by studying variations in the concentrations of glucose, lactate, glutamine, and urea, as well as with the typology of fatty acids contained within triglycerides. Nonspecific responses of the immune system may be perceived from fluctuations in the concentrations of immunoglobulin (Ig)A and IgG and cell dynamics, and may involve direct links to amino acid and protein metabolism. Dysfunctions of the hormonal system may be observed through concentrations of serotonin, cortisol, tes-

Table I. Summary of the biochemical plasma parameters potentially implicated in the occurrence of overtraining in endurance sports[a]

Central parameter	Implied organ	Plasma variation (rest)	Plasma variation (exercise)
Reactive oxygen species	Muscle	CPK ↑; myoglobin ↑; sTi ↑; 3-MTH ↑; retinol (vitamin A) ↓; ascorbic acid (vitamin C) ↓; tocopherol (vitamin E) ↓	CPK ↑; myoglobin ↑; sTi ↑; 3-MTH ↑; MDA ↑; retinol ↓; ascorbic acid ↓; tocopherol ↓
Carbohydrates	Liver, muscle	GLN ↓; urea ↑	GLC ↓; lactate ↑; GLN ↓; urea ↑
BCAA	Body	serotonin ↑	BCAA ↓; fTrp ↑; fTrp/BCAA ↑; serotonin ↑
Glutamine	Muscle, gut	GLN ↓; IgA ↑; IgG ↑	GLN ↑; IgA ↑; IgG ↑
PUFAs	Lymph nodes		PUFAs ↑
Leptin	Adipocytes	Leptin ↓; inhibin B ↓; colecalciferol (vitamin D_3) ↓	Leptin ↓; inhibin B ↓; colecalciferol ↓
Proteins	Muscle, liver, kidney	Haptoglobin ↓; haemoglobin ↑; haemopexin ↓; ferritin ↑; α_1-antitrypsin ↑; α_1-acid glycoprotein ↑; α_2-macroglobulin ↑; T/C ↓	Haptoglobin ↓; haemoglobin ↑; haemopexin ↓; ferritin ↑; α_1-antitrypsin ↑; α_1-acid glycoprotein ↑; α_2-macroglobulin ↑; T/C ↓

a Plasma variations of these parameters are presented as increased (↑) or decreased (↓) during overtraining in athletes well tolerated to hard training.

3-MTH = 3-methylhistidine; **BCAA** = branched-chain amino acids; **CPK** = creatine phosphokinase; **fTrp** = free tryptophan; **GLC** = glucose; **GLN** = glutamine; **Ig** = immunoglobulin; **MDA** = malondialdehyde; **PUFAs** = polyunsaturated fatty acids; **sTi** = skeletal troponine I; **T/C** = free testosterone/cortisol concentration ratio.

tosterone, the T/C ratio, and catecholamines. Also related to the anabolism–catabolism balance of proteins, reactions to different levels of tissue inflammation induced by heavy training may be assessed by plasma kinetics of α_1-antitrypsin, α_1-acid glycoprotein, and α_2-macroglobulin. A possible exercise anaemia may also increase the imbalance of protein turnover, which may be observed through wide variations in haptoglobin, haemopexin, transferrin, and ferritin concentrations. However, the role of exercise-induced plasma volume changes in the interpretation of these type results can no longer be ignored.[73] Substantial biochemical variations in blood become nonsignificant when corrected for plasma volume changes, which have been induced by exercise.

3.2 Perspectives for a Clinical Diagnosis of Overtraining

The main biochemical markers of overtraining remain unpredictable and do not allow their use in a systematic diagnosis of severely overtrained athletes.[5] The nature of training load, as well as training monotony, dietary habits, sleep efficiency, and the psychological status of the endurance-trained athlete also appear as important factors in the occurrence of overtraining.[37] The analysis of all the specific venous blood parameters described in this review remain costly since many necessitate non-routine analytical techniques, and may be subject to significant plasma volume effects. Therefore, another approach is needed to allow longitudinal health monitoring of the athletes engaged in heavy endurance training programmes.[74] While only longitudinal studies monitoring the metabolic response to exercise would be able to discriminate between good and poor training adaptations, the training loads for endurance, sprint, or resistance sport practices may each induce different metabolic, immune, mechanical, neuro-hormonal, and morpho-skeletal adaptations, which may generate different overtraining processes.[8,65] Therefore, while systematic study of the metabolic aspects of overtraining is needed for each sport, attempts to highlight the most relevant biochemical parameters that may reveal overtraining for each individual sport have, as yet, not been successful.

4. Conclusion

The exact aetiology of endurance overtraining is not fully understood but many biochemical parameters appear to allow pertinent information to be documented about the possible occurrence of this chronic fatigue phenomenon. The difficulty is

that none of these metabolic parameters may be considered individually as a standard, allowing a systematic diagnosis of overtraining, or its prevention. This lack of a useful tool in the sport medicine area stigmatises the complexity of the overtraining process. Attempts have recently been made to redefine the overtraining syndrome as the unexplained under-performance syndrome.[75] However, regarding all the parameters that have been linked to overtraining, it appears that only six metabolic schemes have been described in relation to overtraining. A more precise description of each of these metabolic schemes is needed before a more global model of the overtraining process can be developed.

No study has precisely shown the shift from the well-trained state towards the overtrained state in endurance athletes because of methodological difficulties in the longitudinal health monitoring of athletes. Therefore, future research will need to find a global explanation of the steps that lead to overtraining. Currently, variations in energy metabolism appear highly relevant, notably for alterations in carbohydrate and lipid metabolism during training exercises. There is a growing body of evidence that impairment of carbohydrate–lipid metabolism leads to other biochemical aspects of overtraining, i.e. tissue inflammation and protein catabolism, probably in response to metabolic overload in peripheral organs, skeletal muscles and adipose tissue.

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