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Biochemical Aspects of Overtraining in Endurance Sports

The Metabolism Alteration Process Syndrome

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Abstract

Recent studies have shown that endurance overtraining could result from successive and cumulative alterations in metabolism, which become chronic during training. The onset of this process is a biochemical alteration in carbohydrate (saccharide) metabolism. During endurance exercises, the amount of saccharide chains from two blood glycoproteins (α_2-macroglobulin and α_1-acid glycoprotein) was found to have decreased, i.e. concentrations of these proteins remained unchanged but their quality changed. These saccharide chains were probably used for burning liver glycogen stores during exercise. This step was followed by alterations in lipid metabolism. The most relevant aspect of this step was that the mean chain length of blood fatty acids decreased, i.e. the same amount of fatty acids were found within the blood, but overtrained individuals presented shorter fatty acids than well-trained individuals. This suggests that alterations appeared in the liver synthesis of long-chain fatty acids or that higher peroxidation of blood lipoparticles occurred. For the final step of this overtraining process, it was found that these dysfunctions in carbohydrate/lipid metabolism led to the higher use of amino acids, which probably resulted from protein catabolism. The evolution of three protein concentrations (α_1-acid glycoprotein, α_2-macroglobulin and IgG_3) correlated with this amino acid concentration increase, suggesting a specific catabolism of these proteins. At this time only, overtraining was clinically diagnosed through conventional symptoms. Therefore, this process described successive alterations in exercise metabolism that shifted from the main energetic stores of exercise (carbohydrates and lipids) towards molecular pools (proteins) normally not substantially used for the energetic supply of skeletal muscles. Now, a general biochemical model of the overtraining process may be proposed which includes most of the previously identified metabolic hypotheses.

Many parameters have been tentatively used to detect overtraining in endurance sports such as: (i) skeletal muscle cell structural damage;[1] (ii) rest and exercise metabolism alterations;[2] (iii) changes in immunological and/or hormonal response to training stress;[3] and (iv) sympathetic and parasympathetic nervous systems expressions.[4] However, to date, the only reliable criteria for diagnosing overtraining is a loss of performance level despite the maintenance or increase of training load.[5] Nevertheless, a growing body of evidence suggests that various alterations in metabolism may appear during the overtraining process in endurance sports, and that there is a causal link between them.[6] Most of the metabolic pathways have been tentatively studied to highlight overtraining parameters, i.e. carbo-

hydrates,[7,8] lipids,[9,10] amino acids[11] and proteins.[12,13] From all the studies that described the overtraining phenomenon in endurance sports, one key point was that no metabolic process was primarily responsible of its occurrence.[5,6] The second key point was that overtraining is evenly revealed by metabolic parameters related to endurance exercise metabolism, i.e. oxidative processes,[8-10,14,15] or by the consequences of these, i.e. the impairment of immune or hormonal functions.[16-18] Thus, it may be hypothesised that endurance overtraining occurs along metabolism overloads. The real difficulty in determining the occurrence of overtraining is recognising which of these potential parameters will emerge.

In this paper we describe a new theoretical model of the occurrence of overtraining in endurance sports on the basis of experimental data obtained from longitudinal studies. The first part of this paper is devoted to the description of original results obtained from innovative analytical methods. New applications of Fourier-transform infrared (FT-IR) spectrometry were developed to analyse the global metabolic response to exercise in highly endurance-trained individuals.[19] This makes it possible to determine successive alterations in exercise metabolism that lead to the overtrained state. The second part of the paper discusses these results in light of the major models proposed over the last few decades to explain the occurrence of overtraining. The model we present links most of the recent findings regarding overtraining in endurance sports.

1. Investigating Overtraining in Endurance Sports

There is a major methodological discrepancy between most of the studies that tried to analyse the occurrence of overtraining: these studies described clinical and/or metabolic parameters of overtraining from an abrupt increase of training volume and/or intensity.[7,14,20-25] These studies aimed to induce overtraining in a few weeks, generally within 2 or 4 weeks, in order to understand which metabolic consequences of fatigue accumulation correlated the most with overtraining occurrence. In these cases, overtraining manifestations were acute and highly correlated with training load variations (in volume or in intensity). However, most of these studies also found that a complete recovery from overtraining occurred within a space of time no longer than its induction, i.e. only within a few weeks.[14] Thus, the real nature of this apparent overtraining may be questioned since it has also been shown that a complete recovery from overtraining may take several weeks or months of inactivity.[15,21,26] It is possible that the use of a few weeks of hard training load to induce overtraining had instead induced a chronic peripheral fatigue, which is usually rapidly reversible, if not substantially traumatic.[22] In these cases, this important but acute fatigue accumulation should instead be named overreaching.[15,27] Endurance training includes long-lasting exercises performed at submaximal intensities (below the maximal aerobic power), rather than very intense exercises (above maximal aerobic power). To obtain more important metabolic adaptations, the hardness of training load is increased by its volume rather than by intensity. Indeed, the use of a more intensive training load to induce overtraining in endurance sports may be questioned from a methodological point of view, since this intensity increase did not correspond to a normal training stimulus for such athletes. The rapid increase of training volume to induce overtraining in a few weeks is also questionable since no training programme, normally structured, includes such a variation. Doing so ensures a disruption in the training load adaptation process. Several studies reported that overtraining could appear at the end a of training season and while training load had been stabilised for several months.[6,15,28-30] In such circumstances, a complete recovery from overtraining was obtained after several months.[6,15] However, few of these studies reported a sufficiently precise description of the overtraining process to understand the shift from the positive training load adaptations towards the negative ones. The difficulty stems from the problem of monitoring these training adaptations with a sufficient precision, and furthering the progressive disruption of these adaptations, i.e. week-by-week during training. Regarding the biochemical markers that may be considered as potent diagnostic tools in assessing overtraining occurrence,[31] blood samples of about 20–30ml should be taken for each clinical analysis. Thus, for ethical, technical and financial considerations, it is clearly impossible to analyse all these blood parameters each week of the training programme. Therefore, another methodology is needed for the longitudinal health monitoring of athletes.

1.1 Methodology for Longitudinal Health Monitoring of Athletes

During the last few decades, field and laboratory tests have been introduced within training programmes to optimise training load for each athlete. Currently, for endurance sports, several tests are proposed to describe the intrinsic capacities of an athlete, i.e. the determination of maximal oxygen consumption ($\dot{V}O_{2max}$), the energy consumption per motor cycle and the time to exhaustion at maximal aerobic velocity to extrapolate performance level. However, these tests are only performance tests. These are not informative about the health of athletes, and therefore do not allow the prevention or the diagnosis of overtraining.

A wide range of clinical analyses has been proposed to determine athlete fatigue or tissue overload due to intense training load.[31] Recently, a new approach was developed to monitor, week-by-week, the metabolic adaptations to training in highly-trained rowers.[32] A global, and very sensitive, physicochemical analytical method was used, that is FT-IR spectrometry, to analyse plasma contents.[19] Based upon infrared absorptions by organic functions of all biomolecules, plasma FT-IR spectra may be considered as a molecular picture of the whole organic content of plasma (figure 1; table I). This method may be used to determine a wide range of molecular concentrations from capillary blood microsamples, including glucose, lactate, hepatic proteins, immunoglobulins, amino acids, fatty acids, apolipoproteins, cholesterol, triglycerides, glycerol and many more.[33-36] FT-IR spectrometry offers other important advantages in comparison to current clinical analytical methods: (i) the raw analytical data obtained under the form of FT-IR spectra are saved, therefore, plasma contents may be studied at any time that is critical to compare the contents of successive samples and to explain the backgrounds of an emerging physiological situation, such as overtraining; and (ii) the metabolic response to exercise may be determined by subtracting the rest-plasma FT-IR spectrum from the exercise one. Then, a 'difference FT-IR spectrum' is obtained in which only the plasma contents that have been modified by exercise appear.[19] Thus, all exercise-induced plasma biomolecular differences may be studied, even those that were not primarily searched for because of their unknown interest. Finally, on the basis of blood microsamples taken at rest and after a standardised training session performed each week during the training season, this methodology may be used for a step-by-step monitoring of the metabolic adaptations to training load. The convenience of the sampling method, as well as the quality of the results obtained from blood microsamples, provides promising perspectives in sports medicine.[19,37]

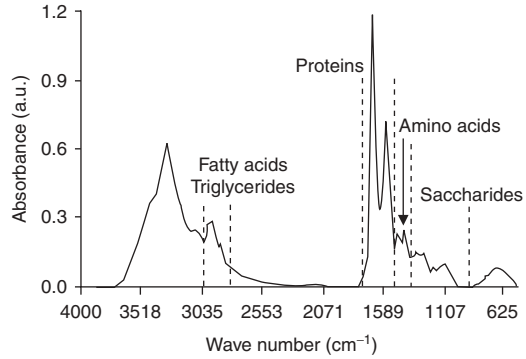

Fig. 1. Characteristic absorption regions of the main biomolecules found within a plasma Fourier-transform infrared spectrum. See table I for details.

Table I. Major assignments of absorption regions observed in plasma spectra

Bands (cm^{-1})	Major assignments for plasma contents
3020–3000	ν = CH (olefinic): unsaturated fatty acids, cholesterol esters
3000–2950	$\nu_{as}CH_3$ (methyl): cholesterol esters, fatty acids, triglycerides
2975–2940	νC-H: glycerol
2950–2880	$\nu_{as}CH_2$ (methylene): fatty acid esters, phospholipids, triglycerides
2880–2860	$\nu_{s}CH_3$ (methyl): cholesterol esters, triglycerides, glycerol
2870–2830	$\nu_{s}CH_2$ (methylene): fatty acid esters, phospholipids, triglycerides
1739–1732	νC = O: lipids, cholesterol esters, triglycerides
1720–1600	νC = O (amide I), β-sheet: proteins
1630–1560	δNH_2 (amine): amino acids
1600–1480	δN-H (amide II): α-helix, proteins δ
1480–1450	$\delta_{s}CH_2$ (methylene): fatty acids, phospholipids
1470–1420	$\delta_{as}CH_3$ (methyl): fatty acids, triglycerides
1430–1360	νCOO^- (carboxylate ions): amino acids, lactate
1300–900	νC-O and νC-O-C: saccharides and carbohydrates; glucose, lactate, glycerol

as = asymmetric; **s** = symmetric; ***v*** = stretching vibrations; δ = bending (scissoring) vibrations.

1.2 Results from the Longitudinal Biological Monitoring of Elite Endurance Athletes

This methodology has been applied to the annual longitudinal monitoring of the metabolic response to a standardised training session (18km at 80% of $\dot{V}O_{2max}$) in 20 elite rowers.[6] For two individuals (we called 'overtrained'), with only small differences in the timing of events, several successive differences were observed in their metabolic response to exercise before overtraining was clinically diagnosed.[6] The first differentiation appeared after 5–7 training weeks on the most specific spectral region of saccharides[38,39] (νC-O absorption; 1300–900 cm^{-1}). However, no significant difference was observed with well-trained individuals for the saccharide concentrations known to evolve during such an exercise, i.e. glucose, lactate and glycerol. Nevertheless, absorption differences were found within the 1300–900 cm^{-1} spectral region, which were at the origin of the differentiation (figure 2). Such spectral differences could be induced only by highly concentrated molecules, i.e. giving intense absorptions potentially found at the surface of the spectrum curve. For plasma contents, only hepatic glycoproteins, apolipoproteins and immunoglobulins contain sufficiently developed saccharide domains within their structure to present such absorptions within the νC-O spectral region.[34]

Using the same plasma FT-IR spectra, concentration measurement and the spectral identification of α_1-antitrypsin, α_1-acid glycoprotein, α_2-macroglobulin, apolipoproteins (A_1, B, C_3) and a wide range of immunoglobulins (A, D, G_1, G_2, G_3, G_4 and M), revealed that only α_1-acid glycoprotein and α_2-macroglobulin presented lower saccharide chain absorptions (figure 3). These reduced absorptions corresponded exactly to the differences found within plasma FT-IR spectra at 1304 and 1257 cm^{-1} (α_1-acid glycoprotein and α_2-macroglobulin, respectively). No other significantly concentrated plasma protein exhibits intense absorption at this spectral location. Indeed, our results revealed that these glycoproteins had 'lost' their saccharide chains. Therefore, it may be hypothesised that this unusual saccharide pool could be used for metabolic requirements within skeletal muscles or liver.

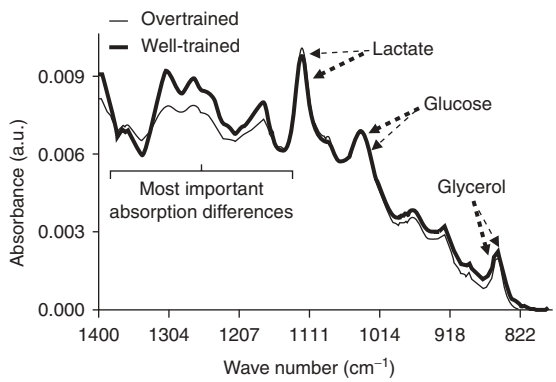

Fig. 2. Absorption differences observed after 5 weeks of training between the exercise-plasma Fourier-transform infrared spectra of overtrained and well-trained rowers (n = 2 and 13, respectively) within the 1300–900 cm^{-1} absorption region.

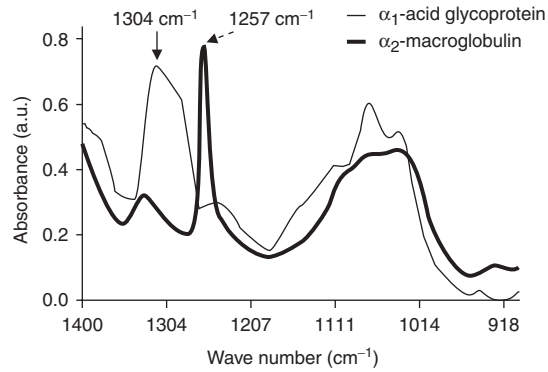

Fig. 3. Absorptions of α_1-acid glycoprotein and α_2-macroglobulin within the 1300–900 cm^{-1} absorption region.

A second differentiation was observed after 8–10 training weeks for the same individuals on the most specific spectral region of cholesterol (ν = CH; 3020–3000 cm^{-1}).[40] Although there was no difference in cholesterol concentrations with well-trained individuals, analysis of plasma FT-IR spectra revealed a significant difference in the repartition between esterified and non-esterified cholesterol fractions (figure 4). Overtrained individuals presented higher concentrations of esterified cholesterol concomitantly with non-significant differences in apolipoprotein (Apo) concentrations, i.e. increases in Apo-A_1 and Apo-B, and a decrease in Apo-C_3. However, the pooled concentration of Apo-A_1 and Apo-B was found to be significantly higher in these individuals. Therefore, the differentiation stemmed from changes in cholesterol transport and compartmentalisation within the body.

The third differentiation appeared after 10–13 training weeks for the same individuals on the most specific spectral region of fatty acids ($\nu_{as}CH_3$ and $\nu_{as}CH_2$; 3000–2950 cm^{-1}),[41,42] but there was no

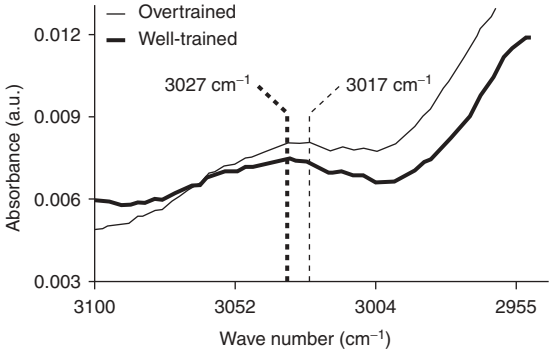

Fig. 4. Absorption differences observed after 8 weeks of training between the exercise-plasma Fourier-transform infrared spectra of overtrained and well-trained rowers (n = 2 and 13, respectively) within the 3100–2950 cm^{-1} absorption region.

difference with well-trained individuals on total fatty acid, free fatty acid and triglyceride concentrations. Nevertheless, differences were observed on these specific fatty acid absorptions (figure 5). On every fatty acid, the CH_2 groups (methylene functions) are intra-chains groups (except for the =CH groups found within unsaturated fatty acids instead of CH_2 groups) while the CH_3 group (methyl function) is the terminal one.[41,43] Indeed, the $\nu_{as}CH_2$ to $\nu_{as}CH_3$ absorption ratio ($\nu_{as}CH_2$**:** $\nu_{as}CH_3$) may be considered as an indicator of fatty acid chain length (but not a direct measurement of chain length since =CH bonds of unsaturated fatty acids are not included in the calculation of this ratio). No quantitative difference could be observed for fatty acid blood contents between well-trained and overtrained individuals since there was no difference in the $\nu_{as}CH_3$ absorption. Therefore, as only the $\nu_{as}CH_2$ absorption was significantly decreased for overtrained individuals, the $\nu_{as}CH_2$**:** $\nu_{as}CH_3$ ratio was also decreased and revealed that fatty acids presented shorter and/or less saturated chains than for well-trained individuals.

While the three differentiations found on saccharide and lipid absorptions were still observed, a fourth one appeared after 13–15 training weeks on carboxylic function of lipids (νC = O; 1751–1729 cm^{-1}). This absorption shifted from 1740 cm^{-1} in well-trained individuals towards 1732 cm^{-1} in overtrained individuals (figure 6). This absorption difference corresponded to the shift found between free and esterified cholesterol fractions.[42] However, the νC = O absorption in plasma FT-IR spectra is not only representative of cholesterol (triglyceride [TG] and phospholipids also present a carboxylic function, and therefore the same absorption). This demonstrates that the differentiation between well-trained and overtrained individuals was substantially increased.

Finally, after 15–18 training weeks, a fifth differentiation appeared on protein (νC = O; amide I; 1720–1600 cm^{-1}) and amino acid absorptions (δNH_2 [amine]; 1630–1560 cm^{-1}). These differentiations revealed that overtrained individuals presented significantly lower protein and higher amino acid blood contents after exercise (figure 7). In parallel, plasma concentration differences were observed with well-trained individuals: (i) higher increases in lactate, glycerol and amino acid concentrations in response to exercise; (ii) significant decreases in glucose and TG concentrations; and (iii) lower protein concentration increases during exercise, which was correlated with the higher amino acid concentration increase. These differences in blood content concentrations appeared concomitantly with the highest differentiation levels we found on the νC-O (1300–900 cm^{-1}; relevant for glucose, lactate and glycerol) and the νC = O (1739–1732 cm^{-1}; relevant for TG) absorptions. Moreover, concentration decreases were found for α_1-acid glycoprotein, Apo-C_3, and IgG_3 which, taken together, were correlated with the amino acid concentration increase. The protein concentration decrease corresponded with 87% of the amino acid concentration increase. These results strongly suggest that α_1-acid glycoprotein, Apo-C_3, and IgG_3 had been cat-

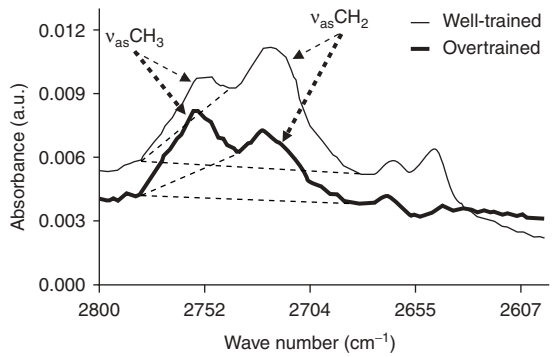

Fig. 5. Absorption differences observed after 10 weeks of training between the exercise-plasma Fourier-transform infrared spectra of overtrained and well-trained rowers (n = 2 and 13, respectively) within the 2800–2600 cm^{-1} absorption region. $\boldsymbol{\nu_{as}CH_3}$ = cholesterol esters, fatty acids, triglycerides; $\boldsymbol{\nu_{as}CH_2}$ = fatty acid esters, phospholipids, triglycerides.

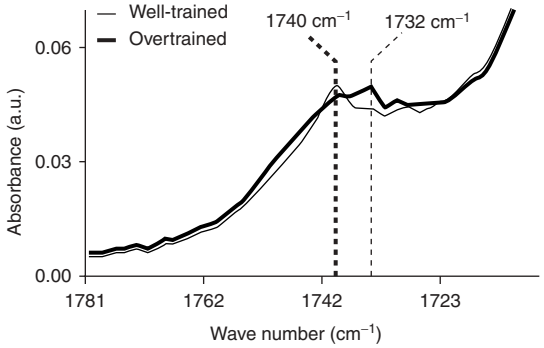

Fig. 6. Absorption differences observed after 13 weeks of training between the exercise-plasma Fourier-transform infrared spectra of overtrained and well-trained rowers (n = 2 and 13, respectively) within the 1780–1680 cm^{-1} absorption region.

abolised to provide amino acids. At this time only, i.e. when differentiations reached protein and amino acid absorptions, the two individuals were clinically diagnosed as overtrained. As well as the drop in performance, individuals complained of tiredness, sleep disturbance, concentration difficulties, muscular pains and were no longer able to finish training sessions at intensities they previously managed.

1.3 The Biochemical Limit Between Overreaching and Overtraining

It is important to note that these alterations in metabolism were successive and cumulative (the first differentiations did not disappear before overtraining diagnosis). The first metabolism alteration appeared for carbohydrates, which was followed by lipids, and finally reached proteins and amino acids. Further, other results provided evidence that the longitudinal monitoring of the metabolic response to a standardised exercise could be used to prevent overtraining: three other individuals avoided overtraining by a 1-week rest period before protein metabolism alteration occurred.[37] All differentiations observed for these individuals (successively: [i] on saccharides νC-O absorption, and [ii] on lipids ν = CH, $\nu_{as}CH_3$, and $\nu_{as}CH_2$ absorptions) corresponded in timing and in chronology exactly with those previously observed for overtrained individuals. However, differentiations disappeared definitively after the 1-week rest period. However, the two overtrained individuals continued to present differentiations during 22 weeks while they trained moderately (half the training programme). These results may be globally paralleled with the findings of other studies, where overreaching obtained after short periods of intensified training (2–4 weeks) necessitated only a few weeks of recovery.[4,27] Inversely, overtraining obtained after several months of hard training necessitated long-term recovery.[11,44] Therefore, it is the chronicity of fatigue accumulation that was related to the level of metabolism alterations.

During exercise, the main biological purpose of carbohydrates and lipids is energy supply to active skeletal muscles. It is the opposite for proteins since these biomolecules have more important biological functions within the body, at rest as well as during exercise.[15,45] Therefore, the possible protein catabolism we found for amino acid supply to skeletal muscles may have reduced biological functions of various proteins. From a metabolic point of view, the limit between overreaching and overtraining is probably between lipid and protein metabolism alterations, i.e. when metabolites other than carbohydrates, lipids and amino acids have to be used for the energy supply to skeletal muscles. Another point of interest for sports biology and medicine is that the clinical diagnosis of overtraining could be given only 10 weeks after onset of this process.

This delay between experimental and clinical symptoms of overtraining may be explained by at least four reasons: (i) the first differentiations observed (differentiations on saccharide chains of glycoproteins and on fatty acid length and/or saturation) did not led to metabolite concentration differences between well-trained and overtrained individuals; (ii) the absorption differences con-

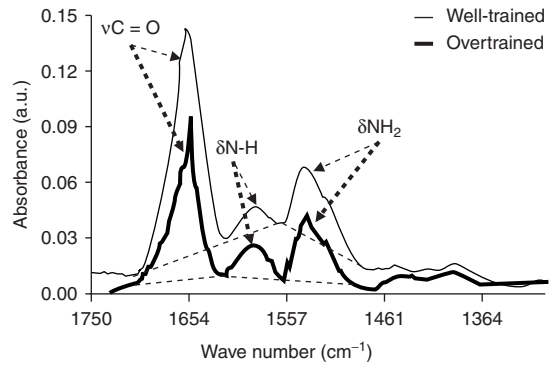

Fig. 7. Absorption differences observed after 15 weeks of training between the exercise-plasma Fourier-transform infrared spectra of overtrained and well-trained rowers (n = 2 and 13, respectively) within the 1750–1300 cm^{-1} absorption region. ***ν*C = O** represents lipids, cholesterol esters, triglycerides; δ**N-H** (amide II) = α-helix, proteins δ; δ$\mathbf{NH_2}$ (amine) = amino acids.

cerned pooled metabolites (saccharide chains of several glycoproteins, total fatty acids, etc.) and could not be correlated with unique concentration changes; (iii) clinical analyses may not highlight changes in undetermined or unknown molecular concentrations while FT-IR spectra revealed all modifications in plasma contents; and (iv) only exercise blood samples revealed significant differences, not the rest samples.

2. The Hypothesis of a Metabolism Alteration Process Syndrome

A lot of metabolic parameters have been proposed to prevent or to diagnose overtraining.[5] All of these have been associated with a chronic fatigue situation in athletes, which may shift from overreaching towards the overtraining syndrome. The limit between overreaching and overtraining has not been clearly pointed out, but a rapid recovery is impossible with overtraining.[15] Indeed, it may be hypothesised that overtraining results from a fatigue accumulation process by which the athlete losses the efficiency of their defence systems.[46,47] For endurance sports, results from several studies suggest that this process might result from alterations in the metabolic response to training stress.[6,8,22,48,49] The results obtained by using FT-IR spectrometry applications during the biological monitoring of athletes have allowed novel insights regarding the alterations in metabolism that may lead to overtraining. A general model may now be proposed in which most of the hypotheses explaining overtraining occurrence, that have been proposed over the last two decades, are included.

2.1 The Saccharide Metabolism Step of Overtraining

It has been shown that impairment of carbohydrate metabolism during intense endurance exercise may contribute to overtraining as a consequence of successive intense training sessions, which induced chronic glycogen depletions within the liver and skeletal muscles.[8] However, glycogen store re-synthesis generally appeared optimal between training sessions. Indeed, it was hypothesised that chronic glycogen store depletions had not induced overtraining *per se*, but rather that this over solicitation of carbohydrate metabolism during exercise could have induced a metabolic fatigue, notably within the liver.[2] By using FT-IR spectrometry for global analyses of plasma, no difference was found between well-trained and overtrained individuals for glucose, lactate and glycerol concentrations, while overtrained individuals were differentiated on the most specific spectral region of saccharides.

In this first step of the overtraining process, we found an absorption decrease for saccharide chains of three glycoproteins (α_1-acid glycoprotein, Apo-C_3 and IgG_3) despite the maintenance of their blood concentration. These proteins may be used as specific glucide donors or under various metabolic stress situations, i.e. hypoglycaemia, metabolic disorders or during long-term fasting.[50] It is well known that plasma has a sugar scavenging capacity through haemoglobin and protein glycosylation (fructosamines), notably for individuals with diabetes mellitus.[51] However, metabolic complications of diabetes may appear by the direct deleterious action of protein sugars, known as glycation or nonenzymatic glycosylation, which may release high amounts of glucose under metabolic stress conditions.[52] It has been shown that stress arising from various work sources may be particularly relevant in the elevation of glycosylated albumin, leading to hyperglycaemia while the concentration of circulating glucose was previously low.[53] However, there is no report of such a mechanism on glucose metabolism in relation to intense endurance exercise and training. Nevertheless, the possible utilisation of saccharide chains of circulating glycoproteins might explain how glycogen stores were found to be more depleted in exercising overtrained individuals, but without evidence of any incomplete restoration between training sessions.[8]

The use of glycoprotein by-products for glycogen store restoration would therefore lower their depletion during exercise and/or it would facilitate their restoration between sessions. Therefore, a first hypothesis in the metabolism alteration process of overtraining is that saccharide chains from various glycoproteins could be used to avoid a chronic glycogen store depletion during endurance exercises. Furthermore, a possible consequence of this saccharide chain utilisation is that the biological functions of these glycoproteins could be reduced or impaired. More specifically, for endurance-trained athletes, this could induce other metabolic stresses within organs, notably an acute phase response to tissue inflammation (α_1-acid glycoprotein and α_2-macroglobulin are acute phase reactants). Nevertheless, these athletes did not consent to a sufficient recov-

ery period from that point and the metabolic stress overload continued to increase.

2.2 The Lipid Metabolism Steps of Overtraining

With the exception of leptin,[9,54] lipid metabolism has not been extensively studied in relation to overtraining in endurance sports. This is very surprising since some of the most important endurance training adaptations occur specifically on lipid metabolism regulation, notably for the sparing of carbohydrate stores during exercise.[55] This lack of data may be explained by the extreme complexity of lipid metabolism regarding exercise and training.[56] Nevertheless, there is little evidence that disorders or down-regulations in lipid metabolism may interfere with the overtraining process. Following the first alteration of exercise metabolism found for saccharides, a differentiation was observed for cholesterol infrared absorption. This differentiation stemmed from changes in the proportions between esterified and free cholesterol within the blood. This result was surprising since endurance exercise acutely reduces cholesterol ester transport in endurance-trained individuals,[57] also reducing total cholesterol blood content.[58,59] In overtrained individuals, total cholesterol remained equal to that of well-trained individuals, but cholesterol ester concentrations increased in overtrained individuals. Therefore, the expected training adaptations on cholesterol metabolism were no longer observed in overtrained individuals.

During oxidative stress, were carbohydrate metabolism is widely increased, low-density lipoprotein (LDL)-cholesterol is much more oxidised.[60] This phenomenon may be over-expressed in the presence of high polyunsaturated fatty acid (PUFA) concentrations.[61] A direct consequence is that cholesterol ester transport and storage within circulating lipoparticles may be increased to compensate for carbohydrate store depletion. Therefore, a metabolic link may be established with exercise oxidative stress, which reduces carbohydrate stores and increases lipid peroxidation, and cholesterol ester transport and storage.[62] This possible mechanism was partially assessed by other results on lipoproteins. In parallel with the differentiation found upon cholesterol absorptions, the pooled concentration of Apo-A_1 and Apo-B was found to have increased in overtrained individuals. These apolipoproteins are the main blood carriers for esterified cholesterol, notably for LDL and high-density lipoparticles.[63] A concentration increase in these apolipoproteins during exercise is therefore synonymous with higher cholesterol ester transport. On the other hand, we observed a slight but not significant concentration decrease of Apo-C_3 after exercise in overtrained individuals. Apo-C_3 is the main lipoprotein for chylomicrons and very low-density lipoparticles that carry TG and fatty acids, rather than cholesterol. This Apo-C_3 is responsible for fatty acid blood transfer from the gastrointestinal tract in the fed state and from the liver in the fasting state.[64] Within the liver, the Apo-C_3 is involved in the regulation of long-chain fatty acid metabolism (LCFA), notably for their transport to peripheral tissues.[65] Along with differentiations in cholesterol absorptions and lipoprotein concentrations (8th week), we did not observe any difference in TG and fatty acid concentrations for overtrained individuals.

However, a few weeks later, a third differentiation appeared with mean fatty acid chain length, as assessed by the decrease in the $\nu_{as}CH_2$**:** $\nu_{as}CH_3$ ratio for overtrained individuals after exercise, but not at rest. The $\nu_{as}CH_2$**:** $\nu_{as}CH_3$ ratio may be considered as an indicator of mean fatty acid chain length and/or saturation.[41-43] Its decrease during exercise without any change in the total fatty acid concentration shows that the liver released much more PUFA and/or less LCFA. PUFA, specifically the n-3 and n-6 series, play a key role in the progression or prevention of human diseases such as obesity and diabetes, mainly by affecting cellular membrane lipid composition, metabolism, signal-transduction pathways and by direct control of gene expression. Dietary PUFA are negative regulators of hepatic lipogenesis that exert their effects primarily at the level of transcription of a number of hepatic lipogenic and glycolytic genes, i.e. fatty acid synthase.[66] Therefore, PUFA regulate the expression of several enzymes involved in carbohydrate and lipid metabolism and the mechanism of control does not require extrahepatic factors or fatty acid metabolism.[46]

In contrast, saturated and monounsaturated fatty acids exert no suppressive action on lipogenic gene expression.[67] The main negative effect of PUFA on lipogenic gene expression is a lowering of biosynthesis in long-chain fatty acids, notably for C18–C24 fatty acids. As a consequence, the LCFA linking to the Apo-C_3 might be reduced and, there-

fore, PUFA might be released in higher amounts out of the liver, lowering the mean chain length of blood fatty acids (and therefore the $\nu_{as}CH_2$: $\nu_{as}CH_3$ ratio). Moreover, when exposed to oxidative stress, blood PUFA can be attacked by free radicals and oxidised into lipid peroxides. The peroxidative breakdown of PUFA involves chain reactions that result in a variety of products such as aldehydes, ketones and cyclic peroxides.[68] These reactions may propagate and modify lipids and proteins, i.e. in cell membranes and lipoproteins that contain PUFA. This peroxidative process is also usually preceded or accompanied by oxidative modification of LDL particle contents.[61] Therefore, oxidative stress overload during endurance training may lower LCFA synthesis and may increase PUFA blood release. This would lead to higher lipid peroxidation, notably for LDL particles, such as cholesterol. Indeed, a link may exist between oxidative stress overload during endurance training, carbohydrate stores depletion, modifications of cholesterol contents in lipoparticles and PUFA negative effects on LCFA synthesis within liver. Taken together, these parameters suggest a global alteration of lipid metabolism due to the accumulation of excessive metabolic stress during training.

After the first hypothesis of the carbohydrate stores scavenging from saccharide chains of glycoproteins, the second step of the overtraining process may be the following: (i) the oxidative stress of exercise-increased cholesterol peroxidation, leading to higher cholesterol ester transport, which was underlined by increases in Apo-A_1 and Apo-B concentrations after exercise as well as by an increase in the cholesterol ester to free cholesterol ratio (but both were not observed at rest); or (ii) further accumulation of oxidative stress leading to an alteration in the TG/fatty acid cycle, possibly by altering LCFA biosynthesis within the liver and by increasing the PUFA concentration, as shown by decreases in Apo-C_3 concentration and $\nu_{as}CH_2$: $\nu_{as}CH_3$ ratio during exercise (but, again, both were not observed at rest).

Although still not fully elucidated, lipid peroxidation (lipoparticles and PUFA) under oxidative stress is thought to be an important mechanism involved in the pathogenesis of inflammation[61] and in immunosuppression.[69] Therefore, there is also a possible link between lipid metabolism alterations and protein markers of overtraining, as observed in our studies.[6]

2.3 The Protein Metabolism Step of Overtraining

While the previous differentiations still increased in intensity (absorption differences between well-trained and overtrained individuals), a final one appeared regarding protein and amino acid absorptions. This result corroborates previous studies were overtraining was clinically revealed by protein or amino acid parameters.[3,11,27,29,49,70] Globally, authors from these studies argued that protein parameters are the most reliable for detecting overtraining occurrence.[15] In our studies,[6] the differentiation between well-trained and overtrained athletes on protein and amino acid absorptions was revealed at the same time as clinical symptoms appeared. We observed that overtrained individuals presented higher amino acid and lower protein blood accumulation in response to exercise than for well-trained individuals. Furthermore, a direct link was found between amino acid blood accumulation and the decrease of three protein concentrations (α_1-acid glycoprotein, Apo-C_3 and IgG_3). This result strongly suggests that proteins were catabolised for amino acid supply during exercise. However, other proteic parameters had previously been included in the overtraining process, i.e. glycoproteins during the 'saccharide step', and apolipoproteins during the 'lipid step'. For glycoproteins (α_1-acid glycoprotein and α_2-macroglobulin), we found that the protidic part of these biomolecules had not been catabolised and that their molar concentration was not different between well-trained and overtrained individuals. Therefore, there was no differentiation on amide I and II absorptions of these biomolecules, but the use of their saccharide chains for carbohydrate store replenishment probably led to impairment of their biological functions. For apolipoproteins, we found concentration variations due to changes in blood lipid contents. However, the concentration increase in Apo-A_1 and Apo-B was counterbalanced by the decrease in Apo-C_3. Indeed, we did not observe any differentiation on amide I and II absorptions during the 'first steps' of the overtraining process.

Two major commentaries arise from these observations: (i) the protein parameters are transient to the overtraining process; and (ii) two glycoproteins (α_1-acid glycoprotein and IgG_3) presented alterations in their metabolism all along the overtraining process, primarily for carbohydrate store scavenging and a possible amino acid blood release after protein

catabolism. Carbon skeletons of several amino acids may be used for tricarboxylic acid (TCA)-cycle metabolism, which is the third most energetic pathway within skeletal muscles, after carbohydrates and fatty acids. An increase in concentration of TCA-cycle intermediates is probably needed to increase the flux of the TCA-cycle and to meet the increased energy demand of exercise. A gradual increase in leucine oxidation subsequently leads to a carbon drain on the TCA-cycle in glycogen-depleted muscles, and may therefore reduce the maximal flux in the TCA-cycle and lead to fatigue. Deamination of amino acids and glutamine synthesis presents alternative anaplerotic mechanisms in glycogen-depleted muscles, but it only occurs at exercise intensities around 40–50% of $\dot{V}O_{2max}$. To date, the importance of this process for endurance exercise metabolism remains unclear.[45] It has been proposed that the maximal flux in the TCA-cycle is reduced in glycogen-depleted muscles due to insufficient TCA-cycle anaplerosis, leading to a limitation in the maximal rate of fatty acid oxidation.[71] It is important to note that amino acid blood accumulation in overtrained individuals corresponded to the highest differentiation levels we found on the νC-O (1300–900 cm^{-1}; relevant for glucose, lactate and glycerol) and the νC = O (1739–1732 cm^{-1}; relevant for TG) absorptions. These differentiations stemmed from a significant glycaemia decrease during exercise, and while lactataemia and glycerolaemia increased. It is also important to note that no differentiation in amino acid and protein absorptions could be observed at rest, underlying this time again that metabolic aspect of overtraining appeared only through exercise metabolism.

It is consequently proposed that the final step of the overtraining process is an impairment of the carbohydrate-lipid metabolism regulation during endurance exercise, which leads to a more abundant use of amino acids, which were probably obtained from catabolised proteins. This utilisation of amino acids may also lead to central fatigue by altering immune and/or hormonal regulations within the body.[15,17,46,48] This might explain the difference in recovery duration after overreaching (few weeks) and overtraining (several months), although overtrained athletes had presented only one more differentiation than overreached athletes: in protein and amino acid metabolism.

3. Conclusions and Directions for the Future

Our studies have demonstrated that an overload in endurance-training may lead to overtraining through successive alterations in exercise metabolism, which become chronic as fatigue increases. Several steps have been clearly identified in this process, which corresponded to successive levels of alterations in metabolism. The process was initialised through carbohydrate metabolism (the most energetic substrate pool for exercise), and followed by lipid metabolism (the most abundant substrate pool of long duration exercise), before reaching protein metabolism (a substrate pool normally not substantially used for exercise). The key step in this process seems to be the shift of alterations in metabolism between lipids and proteins. Several of the major hypotheses that have been proposed to explain overtraining in the past could be directly or indirectly included in the metabolism alteration process we found, namely the carbohydrate hypothesis,[2] the PUFA hypothesis[10,69] and the protein and amino acid metabolism hypothesis.[15,48,49]

We therefore propose redefining overtraining as a metabolism alteration process syndrome rather than the unexplained underperformance syndrome.[72] More work is needed before we can definitively confirm this model regarding specificities of other sport locomotions (walking, running, cycling, swimming, etc.). It is also important to note that all metabolism alterations were revealed in response to exercise, i.e. through the metabolic stress of exercise. Blood contents analysed at rest never revealed any difference between overtrained and well-trained individuals. This difference between rest and exercise blood samples for highlighting overtraining markers probably resulted from the metabolism acceleration obtained during exercise. This may explain, in part, why the results obtained from the clinical analyses usually performed at rest on the fatigued athlete remain unpredictable. Furthermore, most metabolic alterations revealed in this overtraining process resulted from liver dysfunctions (LCFA, PUFA, glycoproteins and apolipoprotein metabolism). Indeed, our results have provided evidence that exercise blood samples are much more useful in detecting alterations in metabolism that reveal the occurrence of overtraining. Future studies on overtraining should analyse the evolution of the metabolic response to exercise during training to

make the distinction between long-term well-tolerated training adaptations and the occurrence of overtraining.

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