



Revisión

Endurance training in fasting conditions: biological adaptations and body weight management

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Abstract

Introduction: in the majority of sports the athlete is required to achieve optimal conditions both at a muscular and metabolic level as well as in body composition, increasing the lean body mass and maintaining a low body fat mass. In this context, different training protocols have been proposed in order to reduce body fat content, by maximizing fat use instead of glycogen.

Objective: to verify if the training while fasting favours the use of fatty acids due to the low glycogen levels, allowing an improvement in the performance and the control of body weight.

Results: protocols have been published, differing in time periods and exercise intensity. In addition, several markers ranging from gene expression analysis to determination of circulating parameters have been assessed in order to interpret the results.

Discussion: at low intensities of endurance-based exercises, adipose tissue lipolysis and muscle fat oxidation rate seem to be higher in fasting than in fed state. On the other hand, glucose metabolism is adapted in order to save glycogen stores, possibly through gluconeogenesis activation. Finally, it has been observed that protein degradation is mainly downregulated. Only one study analyses changes in body composition after fasting during long periods, thus further work is necessary to demonstrate that this is the best method to control body fat.

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Key words: *Fasting. Exercise. Body weight change. Body composition.*

ENTRENAMIENTO AERÓBICO EN AYUNAS: ADAPTACIONES BIOLÓGICAS Y EFECTOS EN EL CONTROL DE PESO

Resumen

Introducción: en la mayoría de las disciplinas deportivas, el deportista debe conseguir unas óptimas condiciones a nivel muscular y metabólico, así como de composición corporal, manteniendo un bajo porcentaje de grasa corporal. En este contexto se han propuesto diferentes protocolos de entrenamiento con el fin de reducir el porcentaje de grasa corporal incidiendo en un aumento de la utilización de las grasas en detrimento del glucógeno.

Objetivo: comprobar si el entrenamiento en ayunas favorece el uso de ácidos grasos debido a los bajos niveles de glucógeno, permitiendo mejoras en el rendimiento y en el control del peso a partir de los estudios publicados.

Resultados: los protocolos publicados difieren tanto en el periodo de trabajo como en la intensidad del ejercicio, así como respecto al análisis de una gran variedad de marcadores, desde la expresión de genes hasta parámetros circulantes.

Discusión: a bajas intensidades de ejercicio aeróbico, los niveles de lipólisis y oxidación de grasas son mayores en el ejercicio en ayunas. Por otro lado, el metabolismo de la glucosa en condiciones de ayuno se adapta en relación al ahorro de las reservas de glucógeno. Finalmente, en condiciones de ayuno, la degradación de proteínas musculares se ve disminuida. Actualmente solo un estudio analiza los cambios en la composición corporal tras un protocolo de larga duración de ejercicio y ayuno, por lo que es necesario realizar más estudios con el fin de demostrar que se trata de una estrategia válida para el control del peso corporal.

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Palabras clave: *Ayuno. Ejercicio. Control de peso. Composición corporal.*

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Introduction

Fats are stored as triglycerides (TGs) in the white adipose tissue in droplets in the vacuoles of adipocytes. This allows of storage of large amounts of energy in small cell volumes. An alternative method for intracellular fat accumulation is offered by brown adipose and in skeletal muscle where TGs can be found in small cytoplasmic lipid droplets. Therefore, white adipose tissue is the principal site for fat storage in humans that can be oxidized during intervals of fasting between meals or during aerobic extensive-based activities (45-55% $\text{VO}_{2\text{max}}$ or even 65% $\text{VO}_{2\text{max}}$ in ultraendurance athletes¹). In both contexts, the objective of fat utilisation seems to be to save muscular and hepatic glycogen stores in order to assure muscular contraction during moments of stress. Therefore, fat utilisation provides, as long as possible, glucose for correct brain function and delaying the use of ketone bodies. However, during exercise performance, fat oxidation can lower during high intensity exercise beyond the so called anaerobic threshold (near 80-90% $\text{VO}_{2\text{max}}$ in athletes), enhancing carbohydrate utilisation².

Therefore, we can conclude that aerobic exercise performance and fasting are two well known strategies that influence lipolysis in adipose and muscle tissue and thereby the amount of body fat mass. This aspect is of utmost importance, since endurance-based athletes could control their body composition in order to establish an optimal balance between lean body mass and fat mass to maximize performance. The question that arises is how efficient would be a combination of both strategies to enhance lipolysis in adipocytes and fat oxidation in skeletal muscle. Otherwise said, how efficient is training while fasting to stimulate lipolysis and control body fat mass in long time endurance training? And, how affect these special conditions in the immune system? Here, we review current studies that address this particular aspect, the implications of the

different energetic metabolic pathways and the results in body weight control.

Objectives

We can address the objective of this review, presenting the accumulated evidence regarding if the combination of both strategies (endurance training and fasting) could help to mobilize fat from the different body stores and which is the role that carbohydrates are playing in this metabolic context.

Results

Fasting state during endurance training

Endurance training is mainly based on aerobic metabolic demands, a situation in which blood lactate production is at the same rate as its degradation disappearance³. Oxidative glycolytic pathway and lipid β -oxidation are used as energetic pathways. Regarding the intensity of exercise bouts, lipids and glucose are used at different percentages⁴.

The protocols used to study exercise practice during fasting periods or glycogen depletion states can be classified as short term (1-2 days) or long term protocols (4-6 weeks) (Table I). In short term protocols, the volunteers undergo a bout of exercise after a fasting period (usually after overnight sleep). In the long term protocols, volunteers perform several training sessions during fasting for several days a week. In these protocols, individuals are monitored by at least 2 tests that are performed at the beginning and at the end of the study during fasting or fed situations. In addition, in these studies, several variables are included regarding the type, intensity and frequency of exercise, as well

Table I
Protocols and duration in the different fasting endurance exercise studies

Study	Duration	Endurance exercise protocols			
5, 6, 7, 9, 12, 13, 16	1-2 days	Fasting state		Exercise protocol	Meal post-exercise
10, 11, 14, 15, 17, 18, 19	4-6 weeks	Pre-test		Diet + Fasting Exercise protocol	Post-test
8	2 days with glycogen depletion in one limb	Glycogen depletion (1 leg)	Meal pre-exercise (fat rich)	Exercise protocol	Meal post-exercise (CHs rich)

as the type of diet followed by the volunteers during the interfasting periods. Nevertheless, the objectives of both types of studies are different. The short term protocols intend to study acute changes in energy metabolism while long term protocols try to find chronic metabolic adaptations (Fig. 1). Finally, other study models include those that compare the response between one limb depleted in glycogen, simulating a low carbohydrate availability, to another non-depleted limb in the same individual.

Adaptations in fat metabolism

When fasting, fat is the major fuel used during exercise. In this case, plasma glycerol and free fatty acids (FFA) levels increase when lipolysis is activated

in adipocytes, which is the first step in using lipids as energy. The activation of this metabolic pathway is carried out by catecholamines binding to beta-adrenergic receptors and by glucagon, and inhibited by insulin. Therefore, circulating glycerol^{5-7,9} and FFA^{5-7,9,12} are used as markers of lipolysis in adipocytes (Table II).

In short term protocols, intensity plays an instrumental role in lipolysis and fat oxidation levels. At low exercise intensity (25-44% of VO_{2max}) glycerol plasma levels increase slightly, but these levels are significantly higher in fasting conditions. Specifically, while fasting, during rest periods glycerol values are 3.5 mmol/kg/min, passing to 8.5 mmol/kg/min after 60 min of exercise, meanwhile in fed states, circulating glycerol values pass from 2.5 mmol/kg/min during rest⁵ to 5.5 mmol/kg/min in the same time interval^{5,7}. These significant differences are maintained at higher exercise

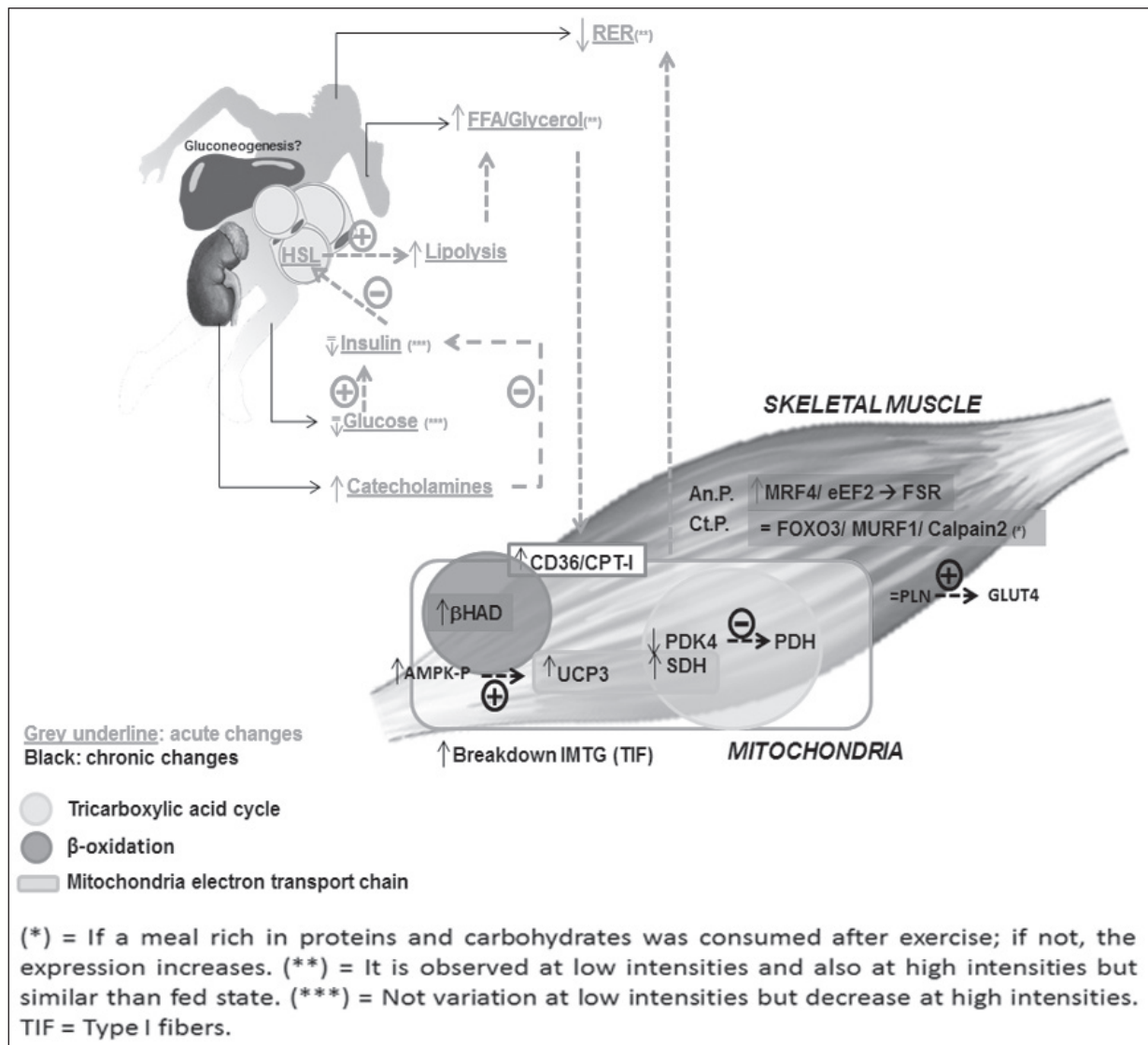


Fig. 1.—Scheme about the different changes in metabolic pathways involved in the fasting-based endurance exercise studies analyzed in the present review. An.P.: protein anabolic pathways; Ct.P.: protein catabolic pathway. (Prepared by the authors).

Table II

Published studies of fasting-based endurance exercise and their results in fasting conditions

Study	N	Subjects	Activity and intensity	Study Design	Results in fasting conditions
5	6	Healthy and active males 26.5 + 3.8 yr. 75.0 + 3.3 kg	Cicloergometre (60min) 44% VO _{2peak}	a) Fed (CHs or CHs+Triglycerides) b) Fasting	↑ Glycerol, FFA, fat oxidation
6	8	Healthy and active males 42.9 + 3.0 yr. BMI 22.5 + 0.4 kg/m ²	Cicloergometre (100min) 50% HR	a) Fed (CHs at 50min exercise) b) Fasting	↑ Glycerol, FFA, adrenaline
7	6	Moderately trained males 25.0 + 2.0 yr. 75.1 + 7.3 kg	Cicloergometre (120min) 25% VO _{2peak}	a) Fed (CHs during exercise) b) Fasting	↑ Glycerol, FFA, fat oxidation ↓ Insulin
			Cicloergometre (120min) 68% VO _{2peak}	a) Fed (CHs during exercise) b) Fasting	↑ Glycerol, FFA ↓ Insulin
8	6	Physically active males 22 to 33 yr. BMI 23.6 kg/m ²	Cicloergometre (150min) 45% VO _{2max}	a) High Fat meal before exercise b) High CHs meal after exercise	↑ UCP3, LPL, PDK4
9	9	Physically active males 22.8 + 0.4 yr. 74.0 + 2.3 kg	Cicloergometre (120min) 75% VO _{2max}	a) Fed (mixed meal) b) Fasting	↑ Glycerol, FFA, fat oxidation, adrenaline, UCP3 ↓ Insulin, RER, IMTG (TIF)
10	20	Physically active males 21.2 + 0.4 yr. 74.8 + 2.0 kg	Cicloergometre (60 to 120min) 171 + 4 W	a) Fed (CHs during exercise) b) Fasting	↑ UCP3, CD36, βHAD, CPTI, SDH (TIIF) ↓ PDK4
11	23	Physically active males a) 20.0 + 1.0 yr. and 79.0 + 7.5 kg b) 21.1 + 1.0 yr. and 74.5 + 7.8 kg c) 20.0 + 1.0 yr. and 77.0 + 10.7 kg	Running (38 min) 90-25%VO _{2max}	a) 1 session/day (4/week) b) 2 sessions/day (2/week) (CHs during exercise) c) 2 sessions/day (2/week)	↑ SDH (in c)
12	8	Healthy and active males 25.0 + 1.0 yr. 74.0 + 3.0 kg	Cicloergometre (60min) 70% VO _{2max}	After exercise in fasting state: a) CHs b) placebo	↑ FFA, MRF4 ↓ MURF1
13	9	Sedentary males 29.0 + 1.0 yr. 75.2 + 1.1 kg BMI 23.6 + 0.3 kg/m ²	Treadmill/ Cicloergometre (45min/ 45min) 60-65%/ 60-65% VO _{2peak}	After fasting state: a) Sedentary + isocaloric + balanced diet b) Exercise + isocaloric + balanced diet c) Exercise + isocaloric + low CH d) Exercise + hypocaloric + low energy	↓ TAG, glycogen (in c)
14	14	Untrained (m) and (f) (m): 29.3 + 4.5 yr. (f): 24.6 + 6.1 yr.	Cicloergometre (25 to 100 min) 65% VO _{2peak}	a) Fed (mixed meal) b) Fasting	↑ CS (in m), glycogen ↓ CS (in f)
15	28	Healthy and active males 21.2 + 0.3 yr. 71.5 + 1.9 kg	Cicloergoemtre/ Treadmill (60min/ 90min) 70-75% VO _{2max} / 85% HR _{max}	Hypercaloric fat rich diet: a) Sedentary b) Fasting state c) Fed	↑ CD36, CPTI, CS (in b and c), glycogen, AMPK-P ↓ FFA (in b and c)
16	8	Trained males 27 + 6 yr. 91.3 + 10.8 kg	Treadmill (36min) 65% HR _{res}	a) Fed (mixed meal) b) Fasting	↑ RER

Table II (cont.)

Published studies of fasting-based endurance exercise and their results in fasting conditions

Study	N	Subjects	Activity and intensity	Study Design	Results in fasting conditions
17	20	Healthy and active males a) 23.0 + 1.1 yr. b) 22.1 + 0.9 yr.	Cicloergometre (60 to 90 min) 70% VO _{2max}	a) Fasting b) Fed (mixed meal)	↑ βHAD, IMTG (TIF), CS, glycogen
18	20	Healthy and active males 21.2 + 0.4 yr. 74.8 + 2.0 kg	Cicloergometre (60 to 120 min) 70% VO _{2peak}	a) Fasting b) Fed (mixed meal)	↑ glycogen ↓ eEF2-P
19	28	Healthy and active males 21.2 + 0.3 yr. 71.5 + 1.9 kg	Cicloergometre/ Treadmill (60 to 90 min) 70-75% VO _{2max} / 85% HR _{max}	Hypercaloric fat rich diet: a) Sedentary b) Fasting state c) Fed	↑ glycogen (b and c), AMPK. AMPK-P, ACCβ-P ↓ PDK4

a), b), c) and d) indicates the different groups used in the trials. BMI: body mass index; (m): male; (f): female; HR: heart rate; CH: carbohydrate; TIF: type I fiber; TIIF: type II fiber; TAG: triacylglycerol; AMPK-P: AMPK phosphorylated eEF2-P: eEF2 phosphorylated; ACCβ-P: ACCβ phosphorylated.

intensities (68-75% VO_{2max}) after 90-95 min of exercise (15 and 12 mmol/kg/min for fast and fed respectively)⁷, reaching at the end of the activity (120 min) the most significant differences (522 and 144 mmol/l of glycerol for fast and fed respectively)⁹. In contrast, in the same study⁷, exercise performed at low intensities (VO_{2max} = 25%) reaches significant differences between fed and fast individuals after 60 min. In another study, the changes in plasma glycerol after 100 min of exercise intensity at 140 w (lower than 65% of VO_{2max}⁹) were not significant between fast and fed states⁶, although the interstitial glycerol levels in adipose tissue were elevated at this time during fasting as well as in the 30 min subsequent to recovery. These results contrast with those previously obtained^{5,7} at low intensities of exercise. One of the differences between both studies was the age of the participants: 42.9 ± 3.0 years⁶ vs 26.5 ± 3.8 and 25.0 ± 2.0 years^{5,7}. In this context, many differences in the hormone levels are present when comparing old vs young individuals, i.e. growth hormone (GH) levels that decrease with age²⁰ and are instrumental in promoting TG hydrolysis^{21,22}. In any case, more studies need to be performed in order to decipher the role of age and as well as other possible variables in this regard.

Plasma FFA levels increase in the same extent than circulating glycerol in individuals performing exercise while fasting. However, the values differ between studies. In low intensity protocols, one study presented significant differences at the beginning (0.15 mM and 0.35 mM in fed and fast states respectively³) and after 60 min of exercise (0.20 mM and 0.45 mM in fed and fast states respectively). However, for a similar intensity of exercise, another study showed no significant differences at time 0 (around 0.2 mM for fed and fast states⁷), but starting to be significant after 70 min of exercise (0.15 mM in fed vs 0.25 mM in fast state). The differences between both studies could be related to the protocol of administration of carbohydrates and

the amount administered to fed individuals. Meanwhile in one study carbohydrates were administered 1h before starting the exercise routine⁵, in the other, carbohydrates were ingested during protocol execution⁷. In the meantime, at moderate or high intensity exercise performances, plasma FFA in fasting conditions start to be significantly higher than in fed conditions after 90 min of exercise: 0.45 mM vs 0.36 mM respectively⁷. High levels of FFA are maintained immediately post-exercise (1.3-1.7 mM)^{6,9} and during the subsequent 2-4 h of recovery (1.8 mM) with no consumption of carbohydrates¹². In this context, it is important to mention that consumption of a carbohydrate-rich meal following 1h of an exercise bout could also lower plasma glycerol levels to normal levels or lower in the first 4h of recovery⁹, while volunteers that only consumed water after a similar exercise bout, maintained elevated glycerol levels longer⁶.

In long term protocols, the complexity of the different intervention designs adds new variables that complicate the interpretation of the results (Table II). For example, in one protocol¹⁵, volunteers trained in fast and fed states during 6 weeks (4 training sessions of 60-90 min per week) and consumed a hypercaloric fat rich diet in resting periods. The plasma FFA levels were determined after a test performed at the beginning and end of the intervention. In addition, carbohydrates were consumed before test execution only in the fed group. One control of sedentary individuals consuming a fat rich diet was included in the study¹⁵. The circulating FFA levels measured after the test performed at the end of the intervention in fast and fed states decreased similarly (0.30 and 0.33 mM respectively) compared with values determined after the test performed at the beginning of the study (around 0.35 mM in fed and fast conditions). However, sedentary control individuals maintained similar FFA levels during the study (0.35 mM)¹⁵. Although the consumption of this type of diet could be controversial, the presented data

seem to indicate that the rate of lipolysis increases with exercise when a fat-rich diet is consumed, although differences between fast and fed group were modest²³. In addition and despite a consumption of carbohydrates before the test in the fed group, the effects of a fat-rich diet tend to suppress the antilipolytic effect exerted by carbohydrates maintaining similar serum FFA levels in fasting and fed groups²⁴.

As previously mentioned, long term protocols allow the measurement of parameters that may give clues about the adaptive response of the organism, such as changes in the pattern of gene expression. In this context, muscle mRNA levels of lipid transporters, such as the membrane protein CD36 and mitochondrial carnitine palmitoyltransferase-I (CPT-I), are highly expressed in muscle cells in fasting situations compared to the fed state after 4h of recovery from the final test in a long term protocol¹⁰ (Table II). However at rest, the expression of such genes varies depending on the studies, some indicating an increase, with a hypercaloric fat rich diet, while others, following a carbohydrate-rich standard diet, demonstrating that there was no significant difference^{10,15}. These apparently discrepant differences between both studies could be due to the administration of a fat-rich diet (50% lipids¹⁵) vs a moderate fat or carbohydrate-rich standard diet (20% lipids¹⁰) during the study period. It has been described that high circulating fat levels after 4 days of a fat-rich diet have the ability of modulating the expression of CD36 and CPT-I genes in a resting situation²⁵, most likely through mechanisms that implicate the inhibition through α_2 adrenergic receptors²⁴. In any case, athletes must be cautious with fat-rich diets during long periods of time, as they are accompanied by a low carbohydrate intake that could affect muscle glycogen homeostasis. In addition, the metabolic and physiologic response varies greatly with those diets, which can affect training capacity²⁶.

On the other hand, the fat oxidation rate (determined through VO_2 and the respiratory exchange ratio (RER)) has been also evaluated both in short/acute and long term protocols with different results. While at low intensities (25-45% $\text{VO}_{2\text{peak}}$) in acute interventions, fat oxidation increased only in the fasting state (5.7-6.8 mmol/kg/min) compared with the fed state (3.1-4.9 mmol/kg/min^{5,7}), no significant changes were observed between fast and fed states at higher intensities (68-75% $\text{VO}_{2\text{peak}}$) being around 10.0-10.5 mmol/kg/min⁷. Long term studies present a similar pattern for fat oxidation than those observed in acute studies for high intensity exercises (0.8-0.9 g of fat oxidized/min¹⁷). Despite this, training improves fat oxidation in each particular condition (fasting and fed) when comparing values obtained at the beginning and end of the study¹⁷. However, both gene expression and activity of the β -oxidation enzyme b-hydroxyacyl coenzyme A dehydrogenase (beta-HAD) increased only after a long time protocol in the fasting situation^{10,17}, suggesting a metabolic adaptation to the fasting state. There-

fore, the question is how these results obtained from gene expression analysis relate with the results of fat oxidation obtained from RER determinations during the tests. First of all and since fat consumption by the volunteers was similar in both studies (20%), an effect of fat coming from diet could be discarded. A likely explanation is that the changes obtained at the gene expression level do not provide a significant increase in the overall pathway of β -oxidation, suggesting that other still non identified mechanisms operate to increase the rate of fat oxidation during exercise.

Nevertheless, other studies have failed to replicate the above mentioned results, observing that there are no significant differences in fat oxidation rates in the test performed at the beginning compared to the test performed at the end of a long term protocol in both fed and fast states¹⁰ (Table II). This could be related to the carbohydrate intake during a moderate or high intensity activity by fed individuals compared with fasting subjects consuming only water during exercise. Carbohydrates can modulate fat oxidation, but the amount consumed during exercise performance in the fed situation could be a key factor. An intake of 0.4-0.8 g of carbohydrates/kg does not change the fat oxidation rate in fed compared to fasting individuals consuming only water^{6,17}. However, a higher dosage (1.5 g/kg) could decrease the fat oxidation rate in the fed situation during exercise to a lesser extent than while fasting (0.5-0.6 vs 0.8-0.9 g/min respectively⁹). Altogether, the most likely explanation could be that at low intensities and for low doses of carbohydrates consumed during exercise, insulin inhibits lipolysis and the glycolytic flux could tend to be the main energetic pathway²⁷. However at high intensities and taking in account the presence of catecholamines in circulation that inhibit the effect of insulin, fat oxidation rate is maintained at the same levels than while fasting, except when high doses of carbohydrates are consumed^{9,28,29}.

However, it is possible that training while fasting may increase lipolysis to levels that surpass fat oxidation rate, therefore limiting the use of this substrate as a main energy source⁵. This provokes a metabolic stress situation that could be solved by uncoupling proton transport to the mitochondrial matrix during oxidative phosphorylation of ATP. The uncoupling in situations of fuel excess is achieved by the induction of mitochondrial uncoupling proteins (UCPs). In this context, UCP-3 belongs to the family of mitochondrial anion carrier proteins (MACP). Fasting in short and long term protocols and situations of glycogen depletion of one limb results in increased UCP-3 expression at the transcription level⁸⁻¹⁰. However in short term protocols, the increased levels of UCP-3 during exercise quickly decrease, returning to basal levels 4h after exercise performance, while after long term protocols low expression levels can be maintained only at rest⁸⁻¹⁰ (Table II). Altogether, this could be interpreted as an adaptation to optimize fat oxidation in cases of high

lipolytic activity, explaining why individuals training in fasting conditions display high levels of lipolysis but the same rate of fat oxidation than fed individuals.

Another aspect to consider is the role of catecholamines. High circulating levels of adrenaline and noradrenaline inhibit the effect of insulin and promote lipolysis and maybe, fat oxidation through hormone-sensitive lipase (HSL) activation in both fast and fed states^{6,9,30}. Fasting athletes while training present higher adrenaline circulating levels during the whole exercise program⁶ as well as afterwards⁹. On the other hand, noradrenaline, instead of adrenaline, could be mostly involved in maintaining lipolysis only in previously fed athletes⁹. In this context, recent studies have shown that the inhibition of insulin and adrenaline release, maintaining noradrenaline levels through the administration of a somatostatin analogue (octreotide), maintains lipolytic levels. However, this picture is more complex, as other regulator molecules such as atrial and brain natriuretic peptides (ANP and BNP respectively) seem to participate in the control of lipolysis under insulin and adrenaline depletion conditions³¹.

In addition to the energy provided by TG stored in adipose tissue, intramuscular triglycerides (IMTGs) have to be taken into account as well. Generally, IMTGs are found as lipid droplets located proximal to mitochondria and sarcoplasmic reticulum³², being more abundant in type I fibres. No changes in IMTG content were observed at the end of an acute exercise bout in fed athletes, while IMTG content in type I fibres decreases when fasting but not in type IIa fibres⁹. Nevertheless, these changes seem to depend on exercise frequency. In long term protocols, frequent exercise sessions per week could significantly increase IMTG breakdown and citrate synthase (CS) activity of type I fibres while fasting¹⁷ (Table II). However, the same type of exercise bout performed less frequently did not present differences between fed and fasted states at the end of the protocol¹⁰. These results coincide with other studies demonstrating that there is a positive correlation between citrate synthase (CS) activity and duration of high intensity exercise, indicating that frequency and duration are key determinants³³. In addition, it has been observed that CS activity also increases when glycogen stores diminish i.e. during a training protocol that combines alternative days of single exercise sessions with days with double sessions³⁴. Altogether, these observations indicate that the frequency of exercise that affects the intramuscular glycogen status could be a determinant factor in IMTG metabolism.

Fat rich diets could favor the storage of IMTG in type I and IIa fibers during long term fasting protocols^{18,19}, although differences between both fibers are not significant. However, intrahepatic lipid stores increase in this situation of fat rich diets³⁵. Several studies have demonstrated that IMTG accumulation is associated with insulin resistance (IR) in obesity and type 2 diabetes^{36,37}. On the other hand, elevated IMTG content does not necessarily reflect a pathological situation, i.e. athletes

show high IMTG contents but a low IR³⁸. In addition, exercise could favor the elimination of lipotoxic intermediates such as long-chain acyl-CoA, diacylglycerols (DGs) and ceramide derived from IMTG metabolism. These metabolites can promote IR through elevations in reactive oxygen species (ROS) and/or impaired insulin signaling through protein kinase C (PKC) activation^{39,40}. Six weeks of hypercaloric fat-rich diet do not cause changes in the DGs and ceramide muscle contents comparing active subjects to sedentary controls¹⁵, indicating that fat excess in diet can counteract the beneficial effects of exercise.

RER can behave differently if training is performed in fed or in fasting states as well as depending on the duration of the intervention (acute or long term). During a 75% VO_{2max} exercise bout, RER while fasting decreased more than in previously-fed athletes⁹. Conversely, during the subsequent 12-24h of recovery, RER decreased more in previously-fed athletes, although in both groups the values remained under 1¹⁶ (Table II). This possibly occurs because meal consumption before exercise performance leads to elevated VO_2 that can be maintained 24 h longer in fed individuals¹⁶. In long term protocols, after a 70% VO_{2max} or 65% VO_{2peak} exercise, 4 and 5 times per week respectively, fast and fed state diminished their RER similarly during the exercise execution^{14,17}, although no information about RER levels post-exercise (12-24h) was provided. Although more studies are necessary to extract clearer conclusions, it seems that acute protocols performed in fasting conditions are instrumental in promoting muscle fat oxidation, but this does not seem to occur in long term protocols.

Adaptations in glucose metabolism

Plasma glucose levels increase after the intake of a meal containing carbohydrates before or during the exercise. Circulating carbohydrates activate insulin release which has the ability of decreasing lipolysis and subsequently fat oxidation through the inhibition of HSL^{6,7,9}. This implies that carbohydrates will be the main source of energy for exercise performance. However, carbohydrate utilisation produces lactate even in exercises performed at low intensity (50% of maximal heart rate (HR)) and in fed state, although glucose circulating concentrations do not reach 2 mM⁶. In short term protocols, fasting state at low exercise intensities (25% VO_{2peak} or 50% HR) maintains plasma glucose levels at 4.8-5 mM during 100-120 min^{6,7}, meanwhile at higher intensities (68% VO_{2peak} or 75% VO_{2max}) glucose levels start to decrease to 4 mM^{7,9}. These changes differ with the observed plasma variations in glycerol and FFA levels, which tend to increase passing from 6.5 mmol of glycerol/kg/min and 0.25 mM FA in 60 min of low intensity exercise to 10 mmol/kg/min and 0.3 mM respectively in 60 min of high intensity exercise⁷. The obvious inter-

pretation is that conditions of high intensity activities while fasting, such as during aerobic exercise, improve the utilization of fats as a fuel due to the drop of plasma glucose levels and risk of glycogen depletion. Consumption of carbohydrate-rich meals during high intensity exercise maintains glucose levels around 5 mM during 90 min. In these conditions, fat levels are maintained at the same levels as while fasting⁷, but saving glycogen and avoiding hypoglycaemia at high intensities of exercise. Several long-term fasting studies demonstrate an increase in saving glycogen stores and higher post-exercise glycogen recovery^{10,14,15,17}, while others do not report such changes¹⁸. This increase has also been reproduced in some short term protocols⁹ but not in others^{8,13}. One of the differences to explain these discrepancies is with regard to the type of diet that the volunteers followed.

On the other hand and after a long term protocol of training while fasting, an exercise test of 60-120 min at 65% VO_{2max} performed at the end of the protocol revealed that plasma glucose was maintained (around 4.75 mM), contrasting with the results obtained in the test performed at the beginning of the protocol in which glucose levels dropped throughout the exercise bout from 4.75 to 3.9 mM¹⁷. Surprisingly, blood glucose levels in previously-fed athletes were reduced from 4.5 to 3.8 mM at the end of both tests in the same type of protocol. This fact could be interpreted as an adaptation to maintain glycaemia through liver gluconeogenesis, avoiding a drop that could cause hypoglycaemia while exercising during fasting conditions. On the other hand, at high exercise intensities (68% VO_{2peak} -75% VO_{2max}) performed in fasting conditions, the drop in plasma glucose values is more prominent, reaching 3.5 and 4 mM after a test of 120 min performed at the beginning of long term protocols respectively^{7,9}. Interestingly, individuals achieve the same adaptive response after 6 weeks of training in fasting conditions by increasing liver gluconeogenesis, therefore maintaining plasma glucose levels at 4.5 mM after 120min of exercise test¹⁷.

Normally, training while fasting does not produce changes in insulin levels at low intensities of exercise (between 25-44% VO_{2peak} ^{5,7}) maintaining plasma insulin values between 5-10 mU/ml. However, at high intensities (between 70-75% VO_{2max}) insulin tends to decrease to 2 mU/ml after one bout of exercise⁹, maintaining those levels 2-4h post exercise (around 5 mU/ml²⁷) (Table II). This is the situation that allows the activation of HSL. Individuals fed with a fat rich diet, but training while fasting over a long period of time, displayed a significant decrease in insulin levels from 38.2 ± 3.5 mU/ml to 27.9 ± 3.3 mU/ml after an exercise test¹⁵. Moreover, it has been reported that fasting in prolonged or acute training protocols following a high fat diet, improves insulin sensitivity^{13,15}, which goes in parallel with the athlete paradox in which trained subjects with high IMTG contents also have a high oxidative capacity³⁸.

The pyruvate dehydrogenase (PDH) complex catalyzes the conversion of pyruvate in mitochondrial acetyl-CoA through an oxidative decarboxylation reaction. This is the key step that links glycolysis with the tricarboxylic acid cycle. In starvation situations, it is necessary to conserve a pool of 3 carbon metabolites, such as pyruvate, to obtain glucose by gluconeogenesis, since metabolic pathways to convert acetyl-CoA in glucose do not exist. The key mechanism to enable gluconeogenesis is the inhibition of PDH by pyruvate dehydrogenase kinase 4 (PDK4⁴¹). While fasting, elevated levels of acetyl-CoA by lipid oxidation can activate PDK4, modulating metabolic fluxes to increase glucose neogenesis⁴². Low levels of PDK4 mRNA during resting periods and after exercise are detected only in a fasting situation after a long term training protocol¹⁰. Similar results are observed in other long term protocols (6 weeks) using a hypercaloric fat-rich diet administered during the resting period. However, after exercise, PDK4 mRNA seems to be increased in the fasting situation but not in a significant manner when compared to sedentary controls or athletes who had not fasted¹⁹. In other reports with acute glycogen depletion in one leg, this limb presented high PDK4 transcription levels just after exercise, although the differences were not significant to those observed in the glycogen-rich limb after 2h of exercise⁸. However, other protocols showed that PDK4 tended to increase^{42,43}, although the experimental conditions were very different to make a clear comparison. Altogether, PDK4 seems to be an instrumental regulator in glucose utilization during exercise in fasting conditions, but additional research is necessary to assess the role of this enzyme^{9,19} (Table II).

Finally, succinate dehydrogenase (SDH), a key intermediate of the tricarboxylic acid cycle and mitochondrial electron transport chain, seems to be modulated by long-term protocols of daily double sessions of intervallic exercise (25-90% VO_{2max}), 2 days at week. This protocol allows starting the second exercise routine with a partial depletion of glycogen reserves. After six weeks of training, SDH activity tends to increase if carbohydrate intake is omitted during the activity¹¹, considering this observation as an optimal adaptation of oxidative metabolism. In addition, the consumption of carbohydrates during the activity does not seem to affect the increase in SDH activity in type I muscle fibers, but in type IIa, SDH increases slightly but not significantly¹⁰. Therefore, adaptations in oxidative metabolism are dependent of carbohydrate consumption and the type of muscle fibers involved.

Modifications in muscle protein

It can be assumed that endurance-based exercise in fasting conditions could enhance muscle protein degradation in order to use amino acids and avoid glycogen depletion. However, certain strategies can prevent this situation. Some studies show that acute exercise whi-

le fasting did not increase the expression of FOXO3 (a key transcription factor in catabolic processes such as apoptosis) nor activation of MURF1 (a member of the muscular ubiquitin-proteasome system) after 2-6 h post-training, provided that a meal rich in proteins and carbohydrates was consumed after exercise¹². However, there are other protein catabolic systems to be considered, such as the Ca²⁺-activated calpains. In this context, calpain 2 increases its expression 2-6 h after an acute exercise bout in fasting conditions if a protein and carbohydrate-rich meal is not consumed just after finishing the training session¹² (Table II). The likely explanation to these observations could be the presence of insulin which increases in circulation when a carbohydrate-rich meal is consumed immediately after exercise. In this manner, insulin promotes inhibition of muscle protein catabolism^{44,45}.

On the other hand, some genes involved in protein synthesis could increase their expression after an acute exercise bout performed while fasting. This occurs independently if a post-exercise meal has been consumed. This is the case of the myogenic regulatory factor 4 (MRF4)¹², member of a transcription factor family that includes Myf5, MyoD and myogenin, all of them involved in the proliferation of satellite cells and their differentiation into myofibers. The analysis of a fractional synthesis rate (FSR) using [²H₅]-phenylalanine as a tracer shows a similar increase in protein synthesis in fed and fast state after exercise¹². Moreover, an elevated expression of heat shock proteins (HSPs) has been detected in the muscles of individuals performing long term protocols (6 weeks) at high training intensities (yo-yo test at 25-90% VO_{2max}) in both fed and fasting situations. Altogether, it seems that the glycogen depletion which accompanies the training fasting period does not seem to promote a clear degradation of muscle proteins, although the role of the different factors involved needs to be deciphered, as well as the role of synthesis and degradation processes¹¹.

In any case, it seems that during acute exercise performance, protein synthesis in the skeletal muscle is blunted⁴⁶, while the increase of intracellular Ca²⁺ is not diverted to calpain activation. Furthermore, Ca²⁺ activates a cascade of signals where Ca²⁺/calmodulin (CaM) acts downstream and modulates Ca²⁺/calmodulin-dependent protein kinase (CaMK), eukaryotic elongation factor 2 kinase (eEF2K), an inhibitor of the ribosomal protein eEF2, and CaMK kinases (CaMKK)⁴⁷. In this situation, Ca²⁺ acts as a second messenger and, together with troponin, is a key piece of the muscle contractile system. On the contrary, in long term protocols, eEF2 is less phosphorylated in fasting than in fed state, therefore increasing protein synthesis, a process that does not seem to be controlled by AMP-kinase (AMPK)^{18,48}.

AMPK is a key regulator of energy metabolism. In this context, when the AMP/ATP ratio increases (i.e. in fasting conditions), CaMKK activates the α -subunit of AMPK in order to reverse the AMP/ATP ratio. This is

achieved through the activation of peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) mediated by Sirtuin 1 (SIRT1)⁴⁹. PGC-1 α activates the use of FFAs by the mitochondria, increasing ATP production. However, despite a daily double session of interval exercise to maintain low glycogen stores and to provoke a high ratio of AMP/ATP, there are no differences between pre- and post-tests in PGC-1 α levels¹¹. Hypercaloric fat-rich diets during long term protocols seem to induce an increase in the phosphorylation of the α -subunit of AMPK after 48h of exercise in fasting conditions¹⁵ (Table II). On the other hand, in long term protocols carbohydrate-rich diets induce a decrease in the levels of AMPK α -subunit phosphorylation during exercise, taking as reference the values observed in the pre-test, while an increase 4h after exercise in both fed and fasting states is usually observed. This is accompanied by a decrease in the phosphorylation of ACC β (substrate of AMPK) with implications in fat metabolism¹⁸. Independently of the type of diet, 6 weeks of exercise in fasting conditions induces high values of AMPK α phosphorylation at rest and during the exercise due to a decrease in ATP levels^{18,19}. In the fed state with a fat-rich diet, AMPK α activation could be inhibited possibly due to an adaptation to use FFAs as the main energy fuel instead of carbohydrates, saving glycogen stores and muscular proteins, allowing training during long periods of time and avoiding a drop of AMP/ATP ratio²³.

To maintain Ca²⁺ homeostasis in muscle contraction during exercise, the sarcoplasmic reticulum (SR) must uptake cytosolic Ca²⁺ through the activation of the sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA). To this end, phospholamban protein (PLN), a downstream target of CaMK-II by phosphorylation of Thr₁₇ and an inhibitor of SERCA, is blunted⁵⁰. At rest, PLN phosphorylation is low but after a long-term exercise protocol, PLN phosphorylation increases 18% in the fed state, while no significant changes are observed in the fasting state¹⁸. The most plausible interpretation is that in long term protocols the basal activity of CaMK-II, especially in fed situations, is regulated. In this context, it has been suggested that the increase of CaMK-II, and thus PLN phosphorylation, indirectly stimulates glucose uptake by GLUT4, favoring insulin sensitivity⁵¹. In other words, training in fed conditions could improve the glucose uptake by the muscle fiber due in part to CaMK-II activity, a situation that does not occur in fasting. Nevertheless, other studies show that glucose uptake in the skeletal muscle through CaMK-II participation is GLUT4 independent⁵². Further studies are necessary to elucidate the major pathways of CaMK-II in the regulation of muscle glucose uptake.

Changes in body composition after training in fasted state

Finally, there is only one study regarding the relation between training while fasting and changes in body

composition¹⁵. The anthropometric analysis showed a significant increase in total body weight after a long term protocol with a hypercaloric fat-rich diet in the sedentary and fed state condition. In contrast, training while fasting allows the athlete to maintain body weight, without changes in their sum skinfolds. Unfortunately, detailed anthropometric studies are missing and the impact of training in fasting conditions to modulate body composition cannot be assessed. Therefore, it is necessary to delve further into these aspects to demonstrate that weight loss is possible while exercising in fasting conditions accompanied by a diet with moderate fat content.

Fasting state and immune system

Endurance exercise while fasting or with low glycogen stores could disturb the immune system since there are evidences that ultraendurance athletes, which train several days per week emulating chronic glycogen depletion, present alterations in lymphocytes' antioxidant status and high levels of oxidative stress⁵³. Notwithstanding of limited evidences, training with low glycogen levels tends to increase the circulating levels of stress hormones and to decrease immune function⁵⁴ and immunoglobulin levels^{55,56}. Therefore and despite that lipolysis could be inhibited at 2-4h post-aerobic exercise^{6,9,12}, carbohydrate rich meals would avoid immune system disturbances⁵⁷.

Discussion

In conclusion and from a metabolic point of view, fasting during endurance training at low (25-44% VO_{2max}) or high (68-77% VO_{2max}) intensity can increase glycerol and FFA blood levels, from the onset of exercise or after 90-95min, provided that a carbohydrate-rich meal is not consumed during the activity. The lipolysis rate during intense workouts is higher than at lower intensities, which is demonstrated by high concentrations of circulating FFA and glycerol (Fig. 1). On the other hand, the rate of fat oxidation is similar in fed as well as in the fasting state at high intensities of exercise, meanwhile at lower intensities, the fasting state could improve fat oxidation rates to a greater extent than in the fed state. Individuals with a low fit could take advantage of a fasting state exercise programme at low intensity that would favour the use of fats as an energy source (corresponding to a RER = 0.7-0.8) and maybe, improve their body composition.

Hepatic gluconeogenesis is an important source of energy during endurance exercise, obtained from amino acids released from the active muscle. This source represents more than 10% of the energy requirements during prolonged exercise in a low muscle glycogen state⁵⁸. It has been observed that protein degradation markers while fasting at high exercise intensities (70%

VO_{2max}) are not activated (i.e. FOXO3, MURF1) nor are sufficient to promote extensive proteolysis (i.e. calpain 2). Under these conditions, protein synthesis-related genes, such as MRF4 or eEF2, are modestly activated. This scenario requires the presence of circulating insulin as a result of a protein and carbohydrate-rich meal. Trained muscles could use more FFA as fuel rather than carbohydrate, while the production of ammonia, a metabolite derived from protein catabolism, seemed to be lower⁵⁸. However, more studies are necessary to prove that low-intensity exercise while fasting does not affect muscle protein status and therefore may be beneficial for low fit individuals.

Finally, regarding muscle adaptations, conditions of low glycogen stores increase IMTG use in type I fibers. Consequently, adipose tissue lipolysis could be increased after exercise to favour IMTG replenishment. In any case, high intensity workouts allow a greater fat oxidation rate than at lower intensities in fasting conditions. Upregulation of mitochondrial UCP-3 while fasting is an adaptive response after a long-term protocol, as well as while resting after 4h of an exercise bout. This allows the adaptation to the lipolysis excess as a consequence of the lesser fat oxidation rate. Genes involved in lipid metabolism increase their expression during a short or long term protocol while fasting. CD36 and CPT-I gene expression and β -oxidation enzymatic activity, such as beta-HAD, correlate with increased levels of muscle β -oxidation even 4h after a high intensity exercise (around 70% VO_{2max}) in fasting conditions (Fig. 1). Diets with high fat contents can upregulate the expression of the above mentioned genes, but further long-term studies are necessary to elucidate the role of these genes in this particular context. On the other hand, glucose metabolism adaptations in the fasting state after a long-term protocol, such as the decreased activation of PDK4 which control glucose flux towards gluconeogenesis, indicates glycogen saving.

Only one study analyses changes in body composition after following a fat-rich diet while fasting in long-term protocols, where the sum of skinfolds was maintained and total body weight did not change. Further studies are necessary to elucidate the advantages of the prolonged effects of exercise while fasting following a balanced diet on body composition in both sedentary populations as well as in athletes.

Conclusions

In conclusion, the experimental evidence does not support the idea that training while fasting during aerobic routines is the best way to optimize metabolic control and body composition by reducing fat mass. In any case and awaiting new studies, the most adequate advice is to train while fasting at low intensities and during the pre-season period for not disturbing a suitable recovery during a program of training load,

especially in ultra-endurance athletes that desire improve the glycogen saving during competition events.

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