Invited critical review

Oxidative mechanisms at rest and during exercise

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Abstract

Carbohydrates (CHO) and lipids provide the amount of energy required for physical and chemical reactions inside the human body. The various constraints the body has to resolve explain the use of these two substrates, catabolized via distinct pathways to one common final reaction. In the classic model, three main organs/tissues for substrate fluxes (liver, adipose tissue and skeletal muscle) and one organ regulating main reactions by adaptation of hormonal secretions (endocrine pancreas) are described. From this point of view, the only interactions between CHO and lipid metabolisms are mediated by glycaemic changes via insulin/glucagon ratio (IGR). However, according to recent advances, this concept seems to have a limited validity as it does take into account neither the many other interactions between CHO and lipid metabolism that are likely to occur in addition to the coarse control by IGR, nor the long-term regulation of energy balance, whose description began with the discovery of leptin. Moreover, it does not include the effects of energy expenditure.

Therefore, this review focuses on three topics: (i) describe interactions between CHO and lipid metabolism at the level of each tissue and organ implied, via hormonal signaling as well as direct action of nutrients, (ii) integrate fluxes of substrates and signals between those tissues at rest in a global view of the metabolism taking into account short-term and long-term regulating factors and (iii) describe separately, to avoid confusion or extrapolation, the short-term and long-term influence of exercise on these regulation loops.

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Keywords: Metabolism; Substrates; Exercise; Skeletal muscle; Adipose tissue; Liver

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Abbreviations: α2R/βR, α2 receptors/β receptors; ACC, acetyl-CoA carboxylase; ALBP, adipocyte–lipid binding protein; AMPK, AMP kinase; ANP, atrial natriuretic peptide; ASP, acetylation-stimulating protein; AT, adipose tissue; AT-LPL, adipose tissue-specific lipoprotein lipase; BNP, brain natriuretic peptide; CHO, carbohydrates; CPT-1, carnitine palmitoyl transferase-1; DAG, diacylglycerol; EGP, endogenous glucose production; ET, endurance training; FABP, fatty acid binding protein; FAT, fatty acid translocase; G6P, glucose-6-phosphate; GLP-1, glucagon-like peptide-1; HSL, hormone-sensitive lipase; IATG, intra-adipocyte triglyceride; IGR, insulin/glucagon ratio; IHTG, intrahepatic triglyceride; IMTG, intramuscular triglyceride; LPL, lipoprotein lipase; NEFA, non-esterified fatty acid; PKC, protein kinase-C; RER, respiratory exchange ratio; SCAT, subcutaneous adipose tissue; TG, triglyceride; VAT, visceral adipose tissue.

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1. Introduction

Species’ survival depends on their ability to cope with environmental constraints. Energy homeostasis, the balance between caloric intake and energy expenditure, is not an exception.

Energy is provided by three nutrients: proteins, carbohydrates (CHO) and lipids, the two latter providing more than 80% of total caloric supply. They enter distinct metabolic pathways producing one common metabolite, acetyl-CoA, which, by mitochondrial oxidation through the Krebs cycle and electron transport in the respiratory chain, results in synthesis of ATP. These substrates fluxes provide the amount of energy required for physical and chemical reactions allowing the whole biological processes.

Any physiological system tends to reach the lowest potential energetic state [1]. So, one can wonder why two distinct substrates, catabolized via distinct pathways to one common final reaction, are indeed used. In fact, the complexity of this system is explained by the various constraints the organism has to resolve.

The first constraint is about energy availability: energy demand is continuous whereas caloric intake is discontinuous. So, mechanisms developed, allowing not only the use of these substrates for ATP synthesis (glycolysis, β-oxidation) but also their storage and releasing (glycogenogenesis, gluconeogenesis, esterification of fatty acids, lipolysis) and even endogenous production of glucose from non-carbohydrate precursors (gluconeogenesis).

The second constraint is about glucose. Most organs and tissues are able to independently use CHO and lipids. However, brain and red blood cells cannot use lipid oxidation and are mainly dependent upon CHO. Therefore lipids tend to be used as the main energy source in order to spare glucose when the latter is poorly available (post-absorptive state) whereas CHO tends to be used when they are abundant (post-prandial state) in order to store lipids whose availability is crucial in the post-absorptive state.

The third constraint is about plasma availability which must be maintained in a range of values allowing survival, too high or too low a rate resulting in short- or long-term complications.

In order to describe factors regulating substrate metabolism, researches focused on identification of tissues and organs where all the reactions take place and where regulating factors are synthesized. In the “classic” conception, three main organs/tissues for substrate fluxes (liver, adipose tissue and skeletal muscle) and one organ regulating main reactions by adaptation of hormonal secretions (endocrine pancreas) are described. According to this model:

(i) The liver is the only organ capable of ensuring constant availability of glucose for it is able to store it (glycogenogenesis), to release it into circulation (glycogenolysis) and to produce it from non-carbohydrate substrates (gluconeogenesis).

(ii) Adipose tissue is considered as a storehouse, capable of ensuring lipid availability, releasing non-esterified fatty acids during the post-absorptive state and storing them after esterification as triglycerides during the post-prandial state.

(iii) Skeletal muscle is the main energy-consumer, using lipids during the post-absorptive state and CHO during the post-
prandial state. It can also store glucose as muscular glycogen to provide energy during exercise, particularly when extra-muscular energy supply does not achieve fast enough energy demand.

In all these tissues, metabolism is modulated by enzymes catalysing limiting steps. The activity of these enzymes is regulated by intermediary metabolites and other factors such as pH or body temperature. But the main factor is certainly the insulin/glucagon ratio (IGR), insulin and glucagons being the two main hormones produced by the endocrine pancreas.

From this point of view, the only interactions between CHO and lipids metabolisms are mediated by IGR, i.e., by glycaemic changes.

In the second half of 20th century, most researches focused on CHO metabolism, opening an « All Glucose » era. Understanding pathogenesis of type 2 diabetes in a therapeutic perspective was the leitmotiv. It is also the time when the concept of insulin resistance was born. One must notice that, although insulin effects on lipid metabolism were already known, insulin resistance has been defined as a defect of insulin action on carbohydrate metabolism [2].

However, in the early sixties, Sir Philip John Randle introduced a concept audacious enough to be the matter of many debates almost half a century later. According to his theory, now demonstrated in resting skeletal muscle, an increase in NEFA availability causes a decrease in muscular carbohydrate utilization [3]. So, he suggested that blood glucose-induced variation in IGR is not the only regulating factor and that many other interactions between CHO and lipid metabolism are likely to occur, anticipating the current concept of nutrient sensing.

Moreover, the “classic” model does not take into account long-term regulation of energy balance, of which the description began with the discovery of leptin. At molecular levels, many advances were made, but their integration in a physiological perspective is not clear yet. So, from our point of view, it seems that, in addition to the coarse control by IGR, many secondary regulation loops ensure an adaptation finest than sole hormonal regulation [4].

Adipose tissue

Adipose tissue (AT) is body’s energy store. The rate of NEFA released into circulation depends on the balance between their esterification and lipolysis. For a long time, it was considered a mere storehouse, releasing or storing NEFA according to the energetic status. However, more precise description of lipolysis, growing knowledge about metabolic heterogeneity of adipocytes according to their topography and, above all, evidence for an endocrine role of AT have made it as the human body’s most voluminous gland.

2. NEFA flux regulation balance between lipolysis and esterification

Lipolysis depends on the balance between lipolytic and antilipolytic systems whose central key enzyme is hormone-sensitive lipase (HSL) activity [4]. It catalyzes hydrolysis of intraadipocyte triglycerides (IATG) to produce NEFA and glycerol. NEFA are transferred to the adipocyte membrane by adipocyte–lipid binding protein (ALBP) whose association to HSL seems crucial to normal lipolysis [5]. NEFA leave through passive or facilitated diffusion via specific carriers whereas glycerol is exported through facilitated diffusion only [6]. ALBP is an enzymatic cofactor of both lipase and esterase activity of HSL, which prevents HSL activity negative feedback from NEFA.

Antilipolytic factors are mainly insulin and catecholamines (via α2-receptors). Lipolytic factors are catecholamines (via β-receptors) and natriuretic peptides ANP and BNP (whose role seems relevant only during exercise) [7]. Other factors were identified but they do not seem physiologically relevant in humans.

NEFA esterification is under control by lipoprotein–lipase (LPL) activity. LPL is a circulating and ubiquitary enzyme catalyzing the hydrolysis of VLDL to produce NEFA. These NEFA enter the cell through passive or facilitated diffusion via fatty acid-binding protein (FABP) or fatty acid translocase (FAT/CD36) [8]. Then, they are activated to acyl-CoA by acyl-CoA synthase. Glucose enters the cell through facilitated diffusion via GLUT-4 transporters and provides glycerol through glycolysis. Three glycerols binding to alcohol function of acyl-CoA results in formation of one TG.

LPL regulation deserves a particular attention: insulin stimulates AT-LPL isofrom (as part of an anabolic pathway resulting in energy storage) and inhibits other tissues’ LPL.

(i) Describe interactions between CHO and lipids metabolism at the level of each implied tissue and organ, via hormonal signalization as well as direct action of nutrients.

(ii) Integrate fluxes of substrates and signals between those tissues at rest in a global view of the metabolism taking into account short-term and long-term regulating factors.

(iii) Describe separately, to avoid confusion or extrapolation, the short-term and long-term influence of exercise on these regulations loops.
isoform (as part of a catabolic pathway resulting in ATP production) [9]. Moreover, more recent studies demonstrated that NEFA plasma rate exerts a negative feedback on AT-LPL isoform [10]. De novo lipogenesis, though, does not seem to be physiologically relevant at rest in human [11].

During the post-absorptive state, NEFA release occurs through HSL activation caused by an increase in catecholamines [12] and a decrease in insulinemia resulting in a reduced LPL activation and, consequently, a reduced NEFA esterification. During the post-prandial state, NEFA release is reduced for HSL is inhibited through an increase in insulinemia [13] whose induces a reduction in the lipolysis-stimulating action of catecholamines [14]. Hyperinsulinaemia also induces NEFA esterification through the activation of AT-LPL isoform.

Besides this fine hormonal regulation, glucose and NEFA plasma rates are likely to have a modulating effect. Negative feedback modulation of AT-LPL isoform activity by NEFA results in a balance between the plasma pools of VLDL and NEFA and the pool of IATG. Recent studies also demonstrate that an increase in blood glucose per se can inhibit whole-body LPL activity [15].

Many points remain to be clarified, however. FABP- and FAT/CD36-dependent transport of NEFA inside adipocytes is still poorly studied. A residual lipolysis in a HSL-deficient mouse model suggests the existence of other lipolytic effectors such as TAG lipase [16], but their relevance in human physiology remains to be established. A paracrine factor named acetylation-stimulating protein (ASP) was also recently discovered. It stimulates glucose uptake and inhibits HSL, favouring NEFA esterification. Its secretion is stimulated by insulin and circulating chylomicrons and also seems correlated to total body fat but in vivo studies of its regulation are needed [17].

Moreover, this is still an incomplete model for it postulates AT to be a homogenous tissue considering metabolism or sensitivity to regulating factors. As a matter of fact, a physiological heterogeneity of adipocytes depending on their topography is now well established.

2.2. Adipose tissue heterogeneity: subcutaneous and visceral adipocytes

In the late sixties, Jean Vague already noticed that metabolic abnormalities in obese patients are linked to an android repartition of AT [18]. Since then, Reaven and others developed this concept which led to the current definition of the metabolic syndrome where waist circumference is considered as the essential criterion [19].

In order to clarify, we distinguish in this review subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT). In fact, differences between superficial and deep SCAT are also described but these differences seem less relevant in a physiological perspective (Fig. 1).

We also limit to processes described in human AT. Various techniques are used, from freshly isolated mature adipocyte to more or less prolonged incubation of AT explants. Each method has its own limits and the available data must be interpreted considering specificities of each protocol.

Three main metabolic differences can be described between SCAT and VAT. First, VAT accounts for 10–15% of total body fat in healthy men [20]. Next, VAT is drained by portal vein directly to the liver whereas a major part of SCAT is drained by systemic circulation. Finally, insulin and catecholamines sensitivity is highly different between visceral and subcutaneous adipocytes.

VAT is less insulin-sensitive and more catecholamine-sensitive than SCAT [21]. As a result, lipolysis is increased and esterification decreased in VAT compared to SCAT [22]. Insulin-induced stimulation of LPL, affinity for ASP and NEFA uptake [23,24] are increased in SCAT compared to VAT. By contrast, insulin-induced glucose uptake and de novo lipogenesis are increased in VAT compared to SCAT [25,26]. This

Fig. 1. Adipose tissue secretions, regulating factors and the VAT/SCAT cycle (NEFA: non-esterified fatty acids; VAT: visceral adipose tissue; SCAT: subcutaneous adipose tissue).
mechanism causes an increase in VAT NEFA esterification compensating the increased lipolysis [27].

This difference is well explained concerning catecholamines and depends on the \( \alpha \)-R (antilipolytic)/\( \beta \)-R (lipolytic) ratio, favouring SCAT lipogenic tendency and VAT lipolytic tendency [28,29].

Then, a SCAT/VAT cycle can be pictured. Lipogenic and antilipogenic SCAT is likely to counterbalance less lipogenic and lipolytic VAT. This balance would depend on the SCAT/VAT ratio and could regulate NEFA flux, the latter not depending on total body fat but on this ratio. Consequently, a significant gain of fat mass would not interfere with NEFA availability if the SCAT/VAT ratio is maintained. If we assume that the level of NEFA flux to the liver or in systemic circulation is associated to metabolic disturbances, then a dissociation of this ratio can cause either protective or pathological effects. In addition, most NEFA released by VAT, drained by portal vein, are taken up by the liver. So, their contribution to the systemic pool of NEFA getting to skeletal muscle seems reduced [12].

2.3. Adipose tissue: an endocrine gland

More than a storehouse, AT plays a key role in metabolism regulation. NEFA have direct effects on hepatic and muscular substrate metabolism and indirect effects through insulin secretion. Those effects have, in turn, consequences on AT metabolism. Therefore, some regulation loops can be pictured. Moreover, the discovery of new regulating factors secreted by AT, such as leptin, drastically changed the classical view of AT as a “mere” storehouse. Since then, many factors were identified (leptin, adiponectin, resistin, IL-6, TNF-\( \alpha \), visfatin, …). Two of them were more intensely studied and are likely to interfere with energy homeostasis: leptin and adiponectin.

Leptin is a circulating hormone almost exclusively produced by AT and acting through the long-chain isoform of its receptor Ob-Rb. It has direct effects on central nervous system, mainly through hypothalamus, inhibiting food intake and increasing energy expenditure, mainly via the autonomic nervous system [30]. Peripheral effects were also described. At the whole-body level, leptin increases insulin sensitivity, enhances glycogen storage and decreases intramuscular and intrahepatic TG pool with a decrease in NEFA esterification and an increase in lipid oxidation [31]. In the liver, it potentiates insulin-induced suppression of endogenous glucose production (EGP) [32]. In skeletal muscle, it increases glucose utilization, thermogenesis and de novo lipogenesis [33,34]. It also directly inhibits insulin secretion [35]. Its secretion is stimulated by insulin, supposedly through the latter’s effect to increase glucose utilization and oxidative glucose metabolism in adipocytes [36] whereas it is inhibited by ghrelin, catecholamines and NEFA [37], with the magnitude of this response depending on total body fat. More noticeable is the “asymmetric” regulation of leptin secretion by food intake: calorie restriction causes a more important decrease in leptin secretion than would be expected in regard to the decrease of total body fat or hormonal responses [38]. So, more than an adiposity regulator, leptin seems to be an under-nutrition-preventing factor.

Adiponectin is an adipocytokine specific of mature adipocyte and is the most abundant transcript [39]. Two isoforms of adiponectin receptor were identified in skeletal muscle and in the liver, respectively [40]. Its plasma rate ranges from 5 to 30 \( \mu \)g/ml and is correlated to insulin sensitivity [40] but, in opposition with leptin, it is negatively correlated with BMI [41]. At the whole-body scale, it increases insulin sensitivity and lipid oxidation [42,43]. In skeletal muscle, it increases lipid oxidation and decreases the IMTG pool [44]. In the liver, it inhibits EGP through potentialization of insulin-induced suppression [40,45] but also through an insulin-independent mechanism. It also inhibits lipid oxidation and decreases the IHTG pool [40,45]. Secretion is heterogeneous considering adipocytes topography. Leptin is strongly correlated to total body fat, although SCAT contributes slightly more to its production than VAT [46]. By contrast, adiponectin is significantly produced by SCAT more than VAT [47].

3. Pancreas

Pancreas has been considered the key regulating organ of CHO and lipid metabolism for a long time since Banting and Best discovered insulin. Its hormonal secretions act as a signal modulating every reaction of substrate metabolism in response to glycaemic variations. A decrease in blood glucose results in a decrease in IGR which stimulates a catabolic pathway of reactions aiming at using energy stores whereas the opposite occurs with an increase in blood glucose.

Molecular mechanisms of insulin secretion were also extensively studied and other short- and long-term modulating factors were recently identified such as NEFA, incretins and adipocytokines. Similar to the balance between NEFA release and uptake by AT, insulin secretion is likely to be regulated by finest mechanisms than the only changes in blood glucose.

Insulin secretion response to NEFA is biphasic. During the post-prandial state, NEFA potentize the effects of glucose on insulin secretion whereas, if NEFA plasma rates are increased beyond 12 to 24 h, insulin secretion is inhibited [48]. In a physiological perspective, this could mean that NEFA induce their own storage through a modulation of insulin secretion. However, during the post-absorptive state characterized by a relative lack of glucose, an increase in NEFA has no effect on insulin secretion in subjects with normal glucose tolerance [49].

Two research teams now seek to describe the mechanisms underlying the effects of NEFA on insulin secretion. The recent discovery of the pancreatic receptor for NEFA called GPR40 could be one of those [50].

Another indirect mechanism could imply the incretin glucagon-like peptide-1 (GLP-1), whose synthesis is stimulated by glucose and lipid binding to gut receptor GPR120 [51]. It is of recent interest to study the role played by intestinal hormones on substrate metabolism and this was emphasized with the therapeutic use of GLP-1 in diabetes [52]. Describing their effects on energy, glucid and lipid homeostasis could result in a better understanding of the link between meal composition and post-prandial metabolism.
At this point, we can already describe a short-term regulation loop between pancreas and AT during the post-prandial state (Fig. 2). NEFA potentiate the blood glucose-induced insulin secretion, which inhibits lipolysis and stimulates LPL (modulated through negative feedback control by NEFA plasma rate). So, the decrease in NEFA causes a decrease in its effect on insulin secretion. There is also a long-term regulation loop between pancreas and AT called adipo-insular axis (Fig. 2). Insulin increases leptin secretion which, in turn, inhibits insulin secretion. Still in a long-term fashion, the lipogenic effect of insulin tending to increase total body fat also contributes to amplify leptin secretion rate. So these two loops act for the same purpose: adapting insulin secretion to total body fat. As the adipo-insular axis is a long-term regulation loop, one can speculate that it also regulates basal leptin concentration which, in turn, influences basal insulin concentration to adapt total energy stores.

4. The liver

Considering that the kidney plays a minor role, the liver is the only organ capable of releasing glucose in systemic circulation in order to ensure availability for brain and for red blood cells. During the post-prandial state, the liver can store glucose as glycogen (glycogenogenesis). During the post-absorptive state, it can release glycogen into circulation as glucose (glycogenolysis) or synthesize glucose from glycogenic or nonglycogenic precursors (gluconeogenesis). Endogenous glucose production (EGP) is the flux of glucose produced and released into circulation from glycogenolysis and gluconeogenesis and it is regulated, according to the classical model, by IGR. EGP regulation was more precisely described during the last 10 years with a focus on various potential regulators such as CHO, lipids, insulin, glucagon, leptin and adiponectin. The concept of EGP autoregulation was also suggested in the early nineties. Based on these elements, the liver also seems to be part of complex regulation loops whose key organ is no longer the pancreas.

4.1. Intrahepatic lipid metabolism

During the post-absorptive state, NEFA are the main substrate for the intrahepatic triglycerides (IHTG) pool. Nearly 30% are taken up by splanchnic tissues and directed toward β-oxidation [53] and, sometimes, ketogenesis. Allosteric regulations inhibit NEFA esterification and de novo lipogenesis: a decrease in citrate and insulin causes a decrease in acetyl-CoA carboxylase-1 (ACC-1) and, thus, a decrease in malonyl-CoA. Consequently, gluconeogenesis is stimulated as an increase in acetyl-CoA and glucagon causes inhibition of pyruvate dehydrogenase.

During the post-prandial state, NEFA are still the main IHTG pool substrate but chylomicrons also have a contribution [54]. De novo lipogenesis is no more physiologically relevant in the liver than in AT. A very flexible system allows the liver to regulate IHTG pool and, consequently, VLDL secretion depending on meal composition. A carbohydrate-rich meal increases de novo lipogenesis whereas contribution of chylomicrons is decreased. The exact opposite is described with a lipid-rich meal. Contribution of NEFA does not change: if the critical increase in insulin following the carbohydrate-rich meal inhibits lipolysis, the decrease in circulating NEFA is compensated through a negative feedback-activation of LPL. The same reaction occurs with a lipid-rich meal [55]. This strong regulation of the NEFA flux getting to the liver is in accordance with their role as regulating factors of liver metabolism.

4.2. Regulation of EGP: an adipo-hepato-insular loop?

EGP is stimulated by glucagon and catecholamines whereas it is inhibited by glucose and insulin. An increase in NEFA results in an increase in EGP if insulin rates are low [56]. Insulin-induced suppression of EGP was demonstrated to result from a predominant decrease in glycogenolysis. Now, if insulin secretion is normal, an increase in NEFA results in an increase in gluconeogenesis and a decrease in glycogenolysis whereas EGP remains equal. So, an increase in NEFA inhibits insulin-suppression of glycogenolysis [57,58]. NEFA effects on liver carbohydrate metabolism lie in their interaction with insulin action. As NEFA also potentialize the effect of glucose on insulin secretion, a short-term regulation loop between AT, pancreas and the liver can be pictured (Fig. 3).

During the post-absorptive state when insulin levels are low, NEFA can stimulate EGP and, in synergy with this flux of glucose, tend to increase insulin secretion. The latter will, in turn, tend to inhibit EGP directly and through insulin-induced down-regulation of lipolysis. Moreover, if blood glucose tends to increase, this tends to decrease EGP.

During the post-prandial state, an increase in blood glucose inhibits EGP and stimulates insulin secretion which, potentialized by a post-prandial increase in NEFA, results in an increase...
in the latter’s esterification and, consequently, in a decrease in their plasma rate and in their inhibiting effect on insulin-induced suppression of EGP.

Bergman and other authors even consider the direct effect of insulin on hepatocytes not fast enough to explain the metabolic changes and suggests that insulin-induced suppression is mediated by the NEFA flux [59]. However, Fisher showed in a model of hepatic insulin-receptor-deficient mouse that insulin-induced suppression of EGP is inhibited despite a decrease in NEFA but a significant role of NEFA in the modulation of this phenomenon cannot be excluded [60]. From our point of view, NEFA and insulin act in synergy and provide a fine-tuning regulation of EGP.

Hepatic autoregulation is thought to be aimed at compensating an inappropriate increase in gluconeogenesis by a decrease in glycogenolysis [61]. In a physiological perspective, it seems rather logical that the organism would spare glycogen if de novo synthesis of glucose is possible. However, if this mechanism was reproduced in vivo, its regulation is still subject to controversy. For a growing number of authors, insulin would be the key regulating factor. As a matter of fact, EGP is increased when insulin levels are low. More recent studies demonstrated that a hypercaloric hyperglucidic diet resulting in an increase in glycogen stores can override hepatic autoregulation [62]. So, the latter appears to be a way to spare glycogen stores more than a fine-tuning regulator of EGP.

4.3. An adipo-hepato-insular loop of long-term regulation

Leptin and adiponectin increase hepatic lipid oxidation, tend to decrease IHTG pool and potentializes insulin-induced suppression of EGP [16,63]. Adiponectin also inhibits EGP in an insulin-independent way [64].

A regulation loop whose purpose would be the modulation of NEFA action and the protection against an increase in total body fat can be pictured (Fig. 4).

If total body fat is low, circulating leptin is expected to be low and circulating adiponectin high. As they both increase hepatic lipid oxidation and decrease EGP, the latter does not tend to stimulate insulin secretion.

Considering increasing total body fat, the proportion between adiponectin and leptin is inverses. During the post-absorptive state, as adiponectin – which has a higher potential of suppressing EGP than leptin – decreases, blood glucose tends to increase more, which results in higher insulin secretion but leptin also inhibits insulin secretion and, thus, its lipogenic effect. During the post-prandial state, when blood glucose and insulinaemia increase, so does leptinaemia. This helps insulin to suppress EGP without too much an increase. This loop, which prevents hyperinsulinaemia, can truly be considered as a protection against a vicious circle of increasing insulinaemia/ increasing body fat.

5. Skeletal muscle

Skeletal muscle is the main energy user of the organism. It takes up 50% of NEFA during the post-absorptive state and 75–80% of glucose during the post-prandial state. So it can be considered as a major regulator of substrate metabolism and their plasma availability.

In healthy subjects, it has a unique ability to use the main substrate (i.e. NEFA during the post-absorptive state and glucose during the post-prandial state) called ‘metabolic flexibility’ [65].

Mechanisms of this flexibility were extensively studied these last 20 years. IGR and substrate availability do not seem to be the major regulating factors as mechanisms of competition were demonstrated to take place inside skeletal muscle.

In their previous article, Randle et al. demonstrated that, in the resting muscle, an increase in lipid oxidation results in a decrease in glucose utilization, opening the debates around the
glucose/fatty acid cycle [3]. About a decade later, Winders and, later, McGarry demonstrated what would become ‘Randle reverse’: high glucose and insulin concentrations can suppress fatty acid oxidation [66].

From now on, we only refer to skeletal muscle as ‘muscle’ but it has to be noticed that some of the reactions studied here are aimed at storing energy for a potential exercise. Physiology of exercising and trained muscle will be reviewed further.

5.1. Substrates flux and storage: glycogen and intramuscular triglycerides

NEFA getting to the muscle come from the plasma pool of NEFA and hydrolysis of VLDL by muscular LPL. They enter the muscular cell using FABP and FAT/CD36 transporters through facilitated diffusion. This flux is highly dependent upon their plasma concentrations [67]. During the post-absorptive state, when their plasma rate is high, 50% of circulating NEFA enter the muscle [68]. During the post-prandial state, insulin stimulates their uptake by FAT/CD36 [8]. However, in the same time, insulin inhibits lipolysis and muscular LPL, significantly decreasing their plasma availability and uptake from VLDL. Thus, this insulin-dependent intramuscular NEFA uptake appears to be irrelevant, at least in the resting muscle.

Once inside, NEFA are activated to fatty acyl-CoA by acyl-CoA synthase. 50% of fatty acyl-CoA are directly oxidized and the other 50% are directed toward esterification and enter the IMTG pool [69]. The latter received growing interest these last few years since evidence of a correlation between size of the intramuscular triglycerides (IMTG) pool and insulin resistance. However, no causal relationship could be established. IMTG are submitted to a relevant and constant turnover estimated at 29 h [69]. Like AT, the size of the IMTG pool depends on the balance between lipolysis and esterification, which is noticeably regulated in normal conditions as fasting-induced increase in NEFA availability has no effect on its size [69]. Other factors are known to influence IMTG content: insulin increases IMTG pool as it inhibits muscular lipolysis and stimulates esterification [70]. Leptin has opposite effects as it stimulates lipolysis and inhibits esterification [30]. In a physiological perspective, the IMTG pool size and turnover rate have no values in themselves. They have to be considered in regard with lipid oxidation rate. As a matter of fact, the ratio between IMTG turnover and lipid oxidation is a significant determinant of intramuscular NEFA availability and concentration. Thus, insulin and leptin would not have such an influence on the latter since insulin simultaneously decreases this turnover and lipid oxidation rate whereas leptin has the exact opposite effect. However, the relative effects of these two hormones on each side of the balance remain to be quantitatively studied. By contrast, catecholamines increase muscular lipolysis without an influence on lipid oxidation [71].

In pathophysiology, an increase in IMTG can be seen as an accumulation resulting from a decreased ability of muscle to oxidize lipids but, in physiology, it can also be seen as an adaptation of muscle to repeated and increased needs in lipids [72].

Glucose enters the muscle mainly through an insulin-dependent mechanism: GLUT-4 translocation to cell surface. It can be potentialized by muscular contraction but this is discussed further as we currently only consider muscle in the resting state. A concentration-dependent transport also exists but he is of minor physiological relevance. Once inside the cell, glucose is phosphorylated to glucose-6-phosphate (G6P) and directed toward muscular glycogenogenesis or toward glycolysis. Glycogenogenesis depends on the balance between glycogen synthase and glycogen phosphorylase. Glycogenogenesis is stimulated by insulin, intracellular G6P concentration, energy demand and intramuscular glycogen concentration. It is aimed at ensuring a sufficient flux of glucose during exercise. It takes place during the post-prandial state when glucose uptake generally exceeds the immediate needs of the resting muscle (excepted after depletion of the glycogen stores due to a recent and intense exercise). Extrapolating this data, one can consider the ability of storing glycogen as another way of regulating blood glucose.

5.2. Intramuscular interactions: Randle upside-down

In a recent review [73], Frayn noticed: “the coarse control of reciprocal utilization of glucose and NEFA in the body is brought about through insulin secretion and fine-tuning is provided by skeletal muscle”. Since Randle’s first publications, these mechanisms have been extensively studied and an elegant system of interactions suggesting that the relationship between carbohydrate and lipid metabolism is indeed reciprocal and independent, even though the latter is now debated (Fig. 5).

The glucose/fatty acid cycle is now well established in vivo in human muscle. To explain the underlying mechanisms, Randle had hypothesized a system of allosteric interactions: excess of acetyl-CoA produced by β-oxidation inhibits pyruvate dehydrogenase and excess of citrate inhibits phosphofructokinase. This results in a decrease in glycolysis, intramyocytosolic accumulation of G6P and, finally, a decrease in hexokinase and in glucose uptake [3]. Nevertheless, recent studies, more than lipid oxidation incriminate NEFA concentration (more precisely acyl-CoA and diacylglycerol (DAG)) which, through activation of an atypical protein kinase-C (PKC), results in serine/threonine phosphorylation of IRS-1 [74]. This causes PI3K-dependent signal inhibition and, thus, inhibits GLUT-4 translocation. So, to summarize, an increased concentration of intramuscular acyl-CoA and DAG causes insulin-dependent glucose uptake inhibition.

Winders’, and later McGarry’s description of ‘Randle reverse’ cycle brought a decisive element to the debate. He demonstrated that an increase in blood glucose and insulinemia decreased hepatic lipid oxidation by inhibiting their intramyocytosondrial transport [66]. Once energy demand is satisfied, glucose in excess is converted to malonyl-CoA by ACC, itself activated by citrate, glucose and insulin. Malonyl-CoA is the main precursor of de novo lipogenesis but it is also a powerful allosteric inhibitor of carnitine palmitoyl transferase-1 (CPT-1) which catalyzes intramyocytosondrial transport of acyl-CoA,
regarded as a key limiting step of lipid metabolism [75] allowing them to enter β-oxidation. Two isoforms of ACC are identified [76]: ACC-1 is predominant in lipogenic tissues and has a cytosolic localization. Cytosolic pool of malonyl-CoA is believed to serve the purpose of de novo lipogenesis [34]. ACC-2, predominant in muscle, has preferential juxtamitochondrial localization close to CPT-1 and malonyl-CoA produced by this isoform is believed to be the key inhibitor of muscular CPT-1, more sensitive to the inhibitor effect of malonyl-CoA than its lipogenic tissues homologue [34]. However, it has to be kept in mind that this inhibiting effect of malonyl-CoA is also observed in the liver and that recent studies demonstrated de novo lipogenesis in muscle [34].

5.3. AMP kinase: at the crossroad of modulations

AMP kinase (AMPK) was named after its ability to be activated in response to energy depletion (an increase in AMP/ATP ratio). To simplify, it stimulates catabolic reactions (leading to ATP production) and inhibits anabolic reactions (leading to ATP utilization). Its inhibiting effect on ACC activation decrease acetyl-CoA conversion to malonyl-CoA and the latter’s inhibition of CPT-1, which leads to an increase in fatty acyl-CoA flux and oxidation into mitochondria [77]. It also inhibits NEFA esterification and de novo lipogenesis [34]. About CHO, it stimulates glucose uptake and glycolysis [77] and inhibits glycogenogenesis with phosphorylation of glycogen synthase [78].

5.4. Leptin, de novo lipogenesis and thermogenesis: not so independent interactions?

These last few years, other studies also demonstrated that AMP-independent mechanisms [79], such as hyperosmotic stress and leptin [80], can also activate AMPK. This suggests that the latter is not only the cell regulator of energy balance. Leptin production is increased during the post-prandial state, stimulated by insulin and glycolysis and its secretion rate is proportional to total body fat. So, it is fully active in situations where substrates (lipid stores and circulating glucose) are abundant.

Leptin and adiponectin potentize insulin-dependent glucose uptake and tend to lower IMTG pool size with an inhibition of NEFA esterification and a stimulation of lipid oxidation. Leptin effects on lipid metabolism need AMPK activation and its effects on glucose metabolism need normal PI3K-dependent signaling [34].

So it seems that leptin plays a role in stimulating thermogenesis. This is well established as well as the fact that these effects are not found after suppression of de novo lipogenesis or lipid oxidation.

According to recent studies by Duloo et al., de novo lipogenesis also occurs in muscle, yet a non-lipogenic tissue. They also demonstrated that hyperglycaemia causes an increase in IMTG and that this increase results from de novo lipogenesis without change in lipid oxidation but with a decrease in glucose uptake and glycogenogenesis [34].

6. From virtuous to vicious circles

At this point, all these data are in accordance with a “modern” vision of substrate metabolism: a coarse control by IGR finely tuned by secondary regulating loops reinforcing catabolic or anabolic effects depending on the nutritional state. Thanks to these loops, the constraints are matched with an optimal management in terms of energy balance.

SCAT/VAT cycle determines a range of plasma NEFA availability. Adipo-insular cycle, through short-term regulation by NEFA or long-term regulation by adipocytokines determines a range of insulinaemia and, thus, leptinaemia. Extending this cycle to the liver as an adipo-hepato-insular regulation loop emphasizes the synergy between IGR and nutrient sensing: in a short-term perspective, it modulates CHO availability depending on NEFA availability and vice versa. In a long-term perspective, it modulates total body fat. All these regulation loops are aimed at modulating substrate availability and, thus,
plasma concentrations. In muscle, Randle’s cycle (the original glucose/fatty acid cycle) and its counterpart, Randle Reverse, act as final regulators of the balance of substrates utilization as they determine metabolic flexibility.

Substrate availability is the major point and this is why the term ‘virtuous circle’ can be used: if the organism considers as a priority to maintain plasma concentrations of these substrates in physiological ranges, it is not only a matter of protection against complications such as hypoglycaemia, atherosclerosis or microangiopathy but also because... physiological concentrations are self-maintaining. In other words, as long as CHO and NEFA concentrations remain in a defined range of values, the organism has a strong ability of regulation but would it fail for a certain amount of time, all these regulation loops would go on playing their role of reinforcing current metabolic conditions as they consider it is strongly ensured by IGR. So, self-maintaining pathological conditions yet just doing their job, they can indeed be named “vicious circles”.

Unfortunately, the finer and more complex the mechanism, the more weak points, the more frequent the breakdown! Many weak points certainly exist. Among them, VAT/SCAT ratio, insulin secretion and leptin sensitivity are the best known. This is linked to the second characteristic of these circles: these are open loops! Independently of genetic mutations, environmental factors can interfere through any of these weak points.

For example (Fig. 6), if the VAT/SCAT ratio is increased, whatever the reason, the increase in NEFA availability causes an increase in insulin secretion directly and through an increase in EGP. NEFA availability can be normalized through hyperinsulinaemia but this favours CHO uptake and utilization in muscle and, consequently, causes a decrease in lipid oxidation during the post-absorptive state. At a given point, this leads to an increase in NEFA plasma concentrations even during the post-prandial state and, then, despite hyperinsulinaemia, muscle uses less CHO than it should, due to glucose/fatty acid cycle and more insulin is needed to maintain blood glucose. Metabolic inflexibility and adipo-hepato-insular-induced hyperinsulinaemia already act as vicious circles. But, if NEFA concentrations remains high for more than 12 h, insulin secretion can be attenuated, though not leading to glucose tolerance trouble but increasing metabolic inflexibility with a net decrease in CHO utilization in the post-prandial state. So, substrate plasma concentrations can still be regulated: the price is hyperinsulinaemia and, unfortunately, its cardiovascular consequences. We will not detail further but consequences are far more serious if insulin secretion cannot match the increasing demand as hyperglycaemia is up to occur, leading to the complete metabolic inflexibility with muscle not using enough NEFA during the post-absorptive state because of hyperglycaemia and not using enough CHO during the post-prandial state because of elevated NEFA concentration and, so, elevated NEFA concentrations and hyperglycaemia are self-maintained. As far as we know, this leads to another well-known vicious circle taking place in the pancreas called glucotoxicity.

To summarize, metabolic flexibility depends on physiological substrate plasma availability and metabolic inflexibility can occur when this availability reaches pathological values. Fortunately, as we said, these are open loops. It means that if, on one side, pathogenic factors can interfere, on the other side, so can therapeutic factors. If metabolic diseases therapy is aimed at restoring metabolic flexibility and physiological substrates concentrations, they could use factors such as nutritional intervention, drugs or physical activity. The second part of this review will be focused on the latter and the mechanisms which can be potentially used to counteract the initial pathogenic factors and turning vicious circles back to virtuous ones.

Fig. 6. A vicious circle: effect of an excess of visceral adipose tissue on an adipo-hepato-insulo-muscular loop of regulation (NEFA: non-esterified fatty acids; VAT: visceral adipose tissue; SCAT: subcutaneous adipose tissue; EGP: endogenous glucose production).
7. Physical activity: breaking vicious circles… or bypassing them?

Physical activity also plays a key role in energy balance regulation as it accounts for about 30% of total energy expenditure.

Studies of physical activity and its effects on metabolism must be cautiously considered. First, a distinction between the effects of acute exercise and repeated exercise (training) inducing long-term adaptations must be made. Exercise must be defined by parameters such as intensity and duration and it includes a time of recovery. A significant repetition of exercises is defined by its frequency and induces long-term adaptations interacting with metabolism during exercise as well as during the resting state. So the body must face new constraints comparing to the resting state.

First constraint is about chemical energy which must be supplied to muscle to be converted into mechanic energy allowing performance.

Second constraint is about blood glucose which must be maintained in ranges allowing both sufficient supply to the muscle and prevention of hypoglycaemia and hyperglycaemia.

Third constraint is about muscular and hepatic glycogen, whose availability is highly correlated to performance and which must be spared as long as possible.

Fourth and last constraint lays on the fact that mobilization of the various energy sources is not equally fast. As energy demand increases and fast supply is needed, the body has to use the most rapidly available substrates.

In addition to these constraints, Nature tends to optimize the energetic cost of these constraints in term of energy cost, which leads to long-term adaptations in order to get a compromise between performance and substrates use.

Considering the data of literature, it is noticeable that they often show discordant results. Two major facts are likely to explain this: first, protocols designed for these studies use different frequencies, durations, intensities, exercise types and subjects are not always matched for their sex, age or training status. Next, diet habits, an important bias, are not always taken into account. Finally, phenomena well established in the resting state are sometimes extrapolated to the exercising state.

Except for particular situations, protein contribution to exercise-induced energy expenditure is negligible. So we will consider CHO and lipids as the two main substrates during exercise. Their respective contribution to total energy expenditure must be finely regulated in order to face the various constraints. We will name this respective contribution substrate balance. Of course, regulating mechanisms differ from the resting state, as do constraints.

So, considering that intertissular interactions and substrates fluxes are, at least partially, different between rest and exercise, one can assume that an interference with regulation loops and vicious/virtuous circles of the resting state can occur during exercise and/or training.

Now, we will focus on the most recent advances concerning this matter. First, we will describe the contribution of each organ and tissue to the metabolic adaptation during exercise with the specific influence of duration and intensity. Next, we will describe the long-term influence of training status on metabolism at rest and during exercise.

8. Exercise

8.1. Same actors playing different parts

Exercise implies circulatory, respiratory, hormonal and metabolic adaptations in order to achieve sufficient supply of energy substrates and oxygen to mitochondria. In the rest of the text, we only consider hormonal and metabolic responses.

Regarding metabolism, exercise has three different stages: starter, exercise itself (with a given duration and various intensities) and recuperation.

CHO used during exercise are blood glucose, muscular and hepatic glycogen and, as described further, lactate. Lipids are plasma NEFA, VLDL, adipocytes TG and IMTG.

Duration and intensity are the two intrinsic parameters of a given exercise which influence the substrate balance. To simplify, the relative contribution of lipids used increases with duration whereas the relative contribution of CHO increases with intensity [81]. Practically, because very intense exercises have a short duration, two main situations are observed: mild and moderate-intensity exercise whose duration can be quite long and short-high-intensity exercise.

Each individual has its own ability in term of exercise intensity, expressed as a percent of maximal oxygen consumption capacity ($V_{O_{2max}}$) which allows a more precise interindividual comparison than absolute performance does. So, it is admitted that intensity is mild under 35% $V_{O_{2max}}$, moderate between 40% and 70% $V_{O_{2max}}$ and high above 75% $V_{O_{2max}}$. Of course, this increase in intensity is a continuum and these values were set arbitrarily. Consequently, studies with the same hypothesis can get two different conclusions if one used 40% $V_{O_{2max}}$ and the other 60% $V_{O_{2max}}$.

One essential constraint, as we already said, is the absolute need to maintain an energetic substrates flux at least equal to their utilization speed rate. Now, aerobic metabolism has a limited capacity, which defines $V_{O_{2max}}$. Beyond a given intensity, substrates oxidation rates do not match energy demand. That is why a system which can produce energy from glycolysis pyruvate exists. This system, capable of producing energy faster than substrate oxidation and in an oxygen-independent way is anaerobic metabolism. Its final metabolite is lactic acid (lactate), whose utilization at rest in negligible (except when tissue hypoxia occurs). Its significant production during high-intensity exercise can induce new interactions considering it is a carbohydrate and a weak acid. Coexistence of aerobic and anaerobic pathways also explains the different muscle fibre types: “slow” type 1 fibres, which are mainly oxidative and “fast” type 2a and 2b fibres, which are mainly glycolytic. Nevertheless, considering the whole muscle, CHO metabolism enzymes are more abundant than lipid metabolism enzymes.

This is one more point about complementarity of substrates. Lipids stores are abundant but their mobilization is slow and they provide energy only through oxidative metabolism.
Glucose stores are less abundant and the constraints of maintaining blood glucose (for survival) and of sparing glycogen stores (for performance) must be matched. However, their mobilization can be achieved faster. Finally, intramuscular substrates can be mobilized faster than extra-muscular ones, whatever their nature.

If we consider all these data, we partially explain the fact that CHO are the main substrate during high-intensity exercise as well as during exercise initiation when energy used for a “starter” effect must be quickly provided. By contrast, the fact that CHO are a minor substrate during mild- and moderate-intensity exercise reflects the organism ability to keep this “safety exit” in case of absolute need and to maintain, as long as possible, the use of a substrate whose plasma concentration does not condition survival and whose stores are the most abundant and not correlated to performance, i.e. lipids.

Now we will discuss the factors regulating substrate balance during exercise and describe how they can allow adaptation to exercise conditions and match the different constraints. Diet habits, nutritional and training status will not be taken into account yet in this part of the text. Influence of the latter will be discussed further.

8.2. An overview

Two mechanisms resulting from exercise seems to initiate metabolic adaptations: muscular contraction and catecholamines production [81]. They cause a decrease in insulin secretion and a variable increase in glucagon production through neural affereces and α-adrenergic action.

Then, energy substrates availability is increased with an increase in EGP and glucose uptake whose synchronization leads to a remarkable maintain in blood glucose. Lipolysis, NEFA uptake and muscular glycogenolysis are also activated. IMTG metabolism during exercise is still an object of controversy.

From this overview, two major points must be pointed out. First, this situation is physiological but the major difference with the resting state is maintained blood glucose and an increase in glucose uptake in spite of a decreased insulin secretion. This implies the existence and action of CHO metabolism regulators other than insulin. Next, the change in balance of substrates during exercise also implies interactions between producing and regulating tissues and a fine-tuning regulation inside muscle.

8.3. Of catecholamines

By which way catecholamine secretion and sympathetic nervous system are activated is still debated and probably multifactorial. Whether this is a nervous signal coming from muscle, or glycaemic changes or anticipation from central nervous system, catecholamines are increased during exercise and are considered a major regulator of metabolic adaptation [81].

They are catabolic hormones, reflecting an increased energy demand. They induce catabolic reactions such as lipolysis in AT (with β-adrenergic signal overtaking α-adrenergic signal) and, more recently demonstrated, in muscle [82]. They also inhibit insulin secretion through α-adrenergic signal [83] and stimulate hepatic and muscular glycogenogenesis. They also have differential effects depending on their concentration. As plasma epinephrine rises, it induces first lipolysis, then glycogenogenesis and finally suppresses insulin action [84]. Norepinephrine stimulates EGP. So, theoretically, they are responsible for substrate mobilization from their various sources, directly as well as acting on pancreatic secretions. However, this has to be balanced with more recent studies taking into account intensity. During mild- or moderate-intensity exercise, catecholamines are poorly increased (up to fourfold the basal rate). So, in this case, they are not expected to have a significant effect on lipolysis and EGP. By contrast, during high-intensity exercise, their rate rises up to 18-fold compared to resting state, which makes them more likely to be physiologically relevant [85].

Moreover, they are quickly secreted is high and their half-life is short. Considering that, during high-intensity exercise, energy must also be quickly supplied, it is logical to find them exerting a direct effect where an indirect signaling pathway would take too much time.

8.4. Pancreas: of insulin secretion blockade

Insulin secretion is decreased during exercise through Langerhans islets sympathetic innervation and increased catecholamines concentration [83]. This causes IGR to decrease which creates a “catabolic” climate where CHO and lipids can be mobilized.

The antilipolytic effect of insulin is decreased and NEFA flux to the muscle is increased, matching muscular energy demand. This decrease in IGR also causes a decrease in EGP suppression and glucose flux to the muscle is increased as well [86].

When exercise duration is high, although plasma NEFA concentration is increased and blood glucose is maintained at a constant rate, insulin secretion remains inhibited. In other words, not only can nutrient-regulation of insulin secretion be overtaken by insulin-secretion inhibiting factors during exercise but other mechanisms, distinct from insulin, can mimic its effects on intramuscular glucose uptake. Nevertheless, glucose still increases insulin secretion as the latter appears less decreased after a carbohydrate intake before or during exercise [87].

The increase in glucagon is significant only during a high-duration or high-intensity exercise. Its regulation seems to depend mainly on glycaemic changes [88].

During recovery, catecholamines rapidly decrease and maintained EGP causes blood glucose to be high, which leads to an increase in insulin secretion and a decrease in glucagon secretion [89,90]. This increase in IGR creates an “anabolic climate” favouring lipids and CHO storing.

As exercise duration and/or intensity increases, the decrease in IGR appears to be linear. Thus, this variable can be considered as a predominant regulation system when intensity is moderate and duration is high. However, during high-
intensity exercise, this decrease is less significant and hardly linear, mainly due to a less marked decrease in insulin secretion [85].

This may be related to our discussion about catecholamines. As long as adrenergic signaling, in conjunction with other factors, can « take enough time » to be mediated through pancreatic hormonal secretions to mobilize substrates which themselves take a significant amount of time to supply energy, this optimal management in term of energy can occur. But whenever energy supply speed rate does not match energy demand, catecholamines are then secreted, exerting less finely regulated but more direct and, thus, faster effects.

8.5. Adipose tissue: of lipids mobilization

Lipolysis is increased during exercise, with NEFA released at a rate two- to fivefold increased compared to basal rate [53]. NEFA esterification is simultaneously inhibited. This can be explained with the decrease in the antilipolytic effect of insulin and the predominantly lipolytic effect of epinephrine. Recent studies have also demonstrated the lipolytic effect of ANP and BNP in animal models and in human in vivo [7,91,92]. Their regulation remains to be studied but their effect is believed to be independent from nutritional status and to compensate a decrease in catecholamines activity [91,92]. Other factors might play a role in HSL regulation during exercise but available data are still unclear and in vivo studies in human are lacking.

AT is not metabolically homogeneous. So, NEFA are mainly provided by SCAT. However, as VAT has an increased lipolytic activity compared to SCAT [21–27], one can hypothesize that it is preferentially depleted during exercise. Moreover, another distinction is now established: lipolysis activity during exercise is higher in abdominal SCAT than in femoral or gluteal SCAT [53]. Duration of exercise influences lipolysis activity: after 4 h of moderate-intensity exercise, it can be increased by tenfold compared to basal rate [93]. By contrast, as intensity progressively increases, lipolysis is unchanged but rate of NEFA released into circulation is decreased. This was attributed to a decrease in AT blood flow due to adrenergic vasoconstriction [94].

So, NEFA availability seems regulated by the synergetic action of catecholamines, insulin and, but it has to be precise, ANP and BNP. But they are not the only lipidic substrate, as VLDL and IMTG are also likely to provide energy to muscle. This point will be discussed further.

8.6. The liver as a store and a source of CHO

EGP is increased during exercise, with a four- to fivefold increase compared to basal state [95]. As the result of glycogenolysis and gluconeogenesis it directly depends on hepatic glycogen stores and gluconeogenic precursor’s availability. It is quite remarkable that, in most situations, EGP is finely correlated to glucose uptake resulting in almost perfectly constant blood glucose.

As exercise duration increases, gluconeogenesis progressively becomes the major contributor to EGP, which can be viewed either as a way to spare glycogen stores or as a reaction to match glycogen depletion.

As exercise intensity increases, i.e., when energy must be quickly provided to mitochondria, glycogenolysis tends back to be the major contributor to EGP. One must notice that, at the very beginning of any exercise, when a fast energy supply is required for a short amount of time, glycogenolysis contribution to EGP predominates over gluconeogenesis.

During recovery, glycogen storage occurs and is correlated to total energy expenditure and glycogen stores levels but regulating factors are still unknown.

Once again, other regulation loops can be pictured. During moderate-intensity exercise, sympathetic nervous system and catecholamines both inhibit insulin secretion, which results in a rise in EGP through a decrease in insulin-induced suppression of gluconeogenesis and glycogenolysis. Catecholamines and decreased insulin secretion, as well as ANP and BNP secretion, result in lipolysis and NEFA flux to the liver tends to increase gluconeogenesis. The resulting increase in blood glucose inhibits EGP directly and through a minor but significant stimulating effect on insulin secretion possibly potentiated by NEFA. However, physiological relevance of this mechanism, significant at rest, remains to be demonstrated during exercise.

As exercise duration increases, glucagon secretion gets to significant levels and, in synergy with NEFA flux, tends to increase gluconeogenesis and decrease glycogenolysis. A carbohydrate-induced increase in insulin secretion before or during exercise tends to decrease EGP. Anyway, lipid and exogenous CHO availability results in hepatic glycogen stores sparing. Most of these effects occur through pancreatic hormones secretions and catecholamines do not play significant role in CHO homeostasis.

During high-intensity exercise, IGR effects do not achieve energy demand anymore and catecholamines, through their important increase, become the main regulators. Marliss et al. showed a catecholamine-induced increase in EGP up to eightfold compared to basal state (with glycogenolysis as major contributor) but, as they inhibit glucose uptake, the latter was only increased three- to fourfold compared to basal state [85]. This resulted in hyperglycaemia. During recovery, the association of a hyperglycaemia with a decrease in catecholamines results in a net increase in insulinaemia. This restores an anabolic climate and, thus, glycogen storage, highly depleted at this intensity.

Glucose homeostasis is well regulated during exercise but the model we described considers muscle as a passive substrates extractor and user depending on extra-muscular signals. In fact, this remarkable coordination between EGP and glucose uptake is not only due to catecholamines, sympathetic nervous system or pancreatic hormones. Recent data suggested that lipid metabolism, intramuscular interactions between CHO and lipids and other factors including IL-6 are part of a “work factor” [96] modulating the balance between EGP and glucose uptake.
At the whole-body scale, hormonal responses and substrates mobilization are influenced by exercise intensity level. The same can be said at the muscle scale. During mild- to moderate-intensity exercise, lipid oxidation is the major catabolic reaction and IGR is the major regulating factor, especially if exercise duration is high. At intensity lower than 30% \( V_{O_2\max} \), energy is almost exclusively provided by plasma NEFA and neither intramuscular substrates nor blood glucose are used. At intensity between 50% and 70% \( V_{O_2\max} \), half the energy is provided by fatty acids (25% from plasma NEFA and 25% from IMTG) and the remaining is provided by CHO (12% from muscular glycogen and 38% from blood glucose). At intensity above 80% \( V_{O_2\max} \), when catecholamine concentrations are high and IGR is less correlated to metabolic changes, 75% energy is provided by CHO (60% from muscular glycogen and 15% from blood glucose) and the remaining is provided by fatty acids [97].

This is in accordance with what we already said: as intensity increases, energy provision switches from plasma to intramuscular sources and from lipids to CHO.

In turn, muscle seems capable to produce a complex signal ensuring an optimal flux of substrate and matching the various constraints. AMPK and lactate production play key roles in this coordination.

After 1 h of exercise at 45% \( V_{O_2\max} \), 60% NEFA released during lipolysis enter muscle, 45% being directly oxidized and 15% being esterified in IMTG pool. These data are influenced by exercise intensity and duration. Like at rest, NEFA uptake is highly dependant on their concentration. However, beyond a given step, this correlation is attenuated, which means that factors other than concentration are likely to limit lipids muscular uptake and utilization [98]. Intramuscular transport and cytosolic translocation are not limiting steps, as demonstrated in various studies [97]. By contrast, CPT-1-dependent intramitochondrial transport of acyl-CoA is a key step [99]. But although malonyl-CoA is decreased during exercise, there seems to be a lack of correlation between its concentration and in CPT-1 activity [100]. AMPK inhibits ACC-2, which catalyzes malonyl-CoA synthesis from acetyl-CoA but AMPK seems to play a significant role only during deep glycogen depletion or prolonged exercise [101]. So malonyl-CoA is not likely to be the key regulating factor of CPT-1 during exercise.

CPT-1 is also regulated by intramuscular rate of its main cofactor, carnitine. Studies at rest did not find a correlation between intramuscular carnitine concentration and lipid oxidation rate [102]. However, during high-intensity exercise, an increase in glucose utilization results, through PDH activity, to an increase in acetyl-CoA and in acetyl-carnitine/free-carnitine ratio (AC/FC) [103]. This AC/FC ratio is correlated to RER and negatively correlated to CPT-1 activity and lipid oxidation rate. As glycogen depletion gets deeper, an increase in AMPK activity, resulting in an increase in malonyl-CoA concentration, is likely to reinforce the AC/FC ratio inhibiting effect on CPT-1 activity. By contrast, during moderate-intensity exercise, especially if it is prolonged, intramuscular CHO metabolism is decreased resulting in a decrease in AC/FC ratio and, thus, in CPT-1 inhibition, which allows lipid oxidation. Although mechanisms are different, this can be viewed as an equivalent of the ‘Randle Reverse’ during exercise: an increase in CHO utilization results in a decrease in lipid oxidation (Fig. 7).

IMTG metabolism during exercise is still extensively studied. Unfortunately, the many differences in subjects recruitment, exercise paradigm and AT study techniques limit their interpretation. Nutritional status influences the IMTG pool size as it is increased by fat-rich diet and decreased by CHO-rich diet [97]. IMTG are usually found close to mitochondria, which

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**Fig. 7.** Intramuscular substrate interactions during exercise, reproduced from Kiens et al. (CL: citrate lyase; ACC: acetyl-CoA carboxylase; AMPK: AMP-kinase; PDH: pyruvate dehydrogenase; CPT: carnitine palmitoyl transferase; CAT: carnitine acyl transferase).
suggests that they might play a role similar to glycogen as an immediately available substrate. In fact, it seems that training status, more than the exercise paradigm, influences IMTG utilization during exercise [104]. In most studies, IMTG pool size does not change after a single bout of exercise [53]. During moderate-intensity exercise, the effects of decreased insulinaemia and increased lipid oxidation on IMTG pool are compensated by the NEFA flux. During high-intensity exercise, lipid oxidation is decreased and increased lactate concentration inhibits NEFA uptake. Catecholamines stimulate muscular lipolysis, which seems paradoxical. However, metabolism during recovery should be taken into account as it tends to replenish substrate stores proportionally to energy depletion. Whatever the hypotheses, studies without nutritional bias using techniques directly measuring IMTG turnover are crucial to our understanding of IMTG roles during exercise.

IMTG and NEFA are not the only lipid substrates but VL DL s were so far considered a negligible energy source. However if this postulate were wrong, IMTG contribution would be overestimated. Currently, recent studies have demonstrated that VL DL processing is increased after 1 h of recovery and that this processing rate was influenced by exercise intensity and duration, and by the nutritional status (this study used fat-rich diet) [105]. In fact, VL DL seem to have a significant role during recovery. VL DL–NEFA uptake is under the control of muscular LPL and despite an increase in insulinaemia during recovery, LPL activity is increased in proportion to energy expenditure [106]. LPL activity is maximal 18 h after the end of exercise, which coincides with IMTG pool size nadir and is also influenced by exercise duration and intensity [107]. Therefore, two similar phenomena occur during exercise and recovery which demonstrate why the various regulation loops can be qualified as ‘open’. During exercise, an increased muscular glucose uptake coexists with a decreased insulinaemia as well as during recovery, an increased muscular VL DL–NEFA uptake coexists with an increased insulinaemia. So, if an increase in insulinaemia is crucial to redirect blood glucose toward glycogenogenesis in order to replenish glycogen stores, the remaining glucose must match the vital constraint of maintaining blood glucose and cannot be used as energy source for the rest of the body. Fatty acids would be expected to supply the energy glucose cannot and, as an increase in insulinaemia inhibits lipolysis, this apparently paradoxical increase in LPL activity, with mechanisms overriding insulin effects on LPL activity, could allow NEFA–VL DL uptake and their use as energy source.

Factors regulating CHO metabolism during exercise are also different compared to the resting state. Insulin secretion is inhibited, although blood glucose still has a small but significant effect. The major fact is GLUT-4 translocation occurring through an insulin-independent pathway [108–110].

Muscular glycogen is another source of CHO and performance is conditioned by its disposal. There seems to be no evidence for glucagon receptors in muscle. Glucose uptake and utilization appears to be under the control of AMPK, activated when AMP/ATP ratio increases or when muscular contraction and glycogen depletion occur [76].

During moderate-intensity exercise, NEFA flux achieves energy demand, sparing glycogen stores. In this case, muscular contraction is the only factor activating AMPK, and its stimulation of glucose uptake and utilization is reduced.

However, as intensity is increased, muscular glycogen is degraded in response to catecholamines and, as energy demand is increased, AMP/ATP ratio gets higher. AMPK activation is highly stimulated, resulting in an increase in glucose uptake and utilization whereas accumulation of pyruvate and acetyl-CoA tends to inhibit lipid oxidation through an increase in AC/FC ratio.

Moreover, at a given intensity termed ‘lactate threshold’, anaerobic metabolism contribution to energy supply becomes significant [111]. This is not due, as previously believed, to a ‘Pasteur-like effect’ (i.e. oxygen supply can no longer achieve mitochondrial needs), but rather to an unbalance between metabolic pathways [112]. Thus oxidative pathways can no longer process all the pyruvate produced by carbohydrate breakdown and pyruvate is thus directed toward anaerobic conversion to lactate. Lactate itself can be either oxidized or recycled as a gluconeogenic precursor into the liver (Cori cycle). An increase in lactate concentration also results in metabolic acidosis which inhibits lipolysis and muscular NEFA uptake. In fact, there is a basal lactate production and its increase starts from the beginning of exercise but, under the lactate threshold, its oxidation rate matches its production rate. From the lactate threshold, however, production predominates over oxidation, resulting in an increase in lactate concentration [111]. As a result of all these interactions, during high-intensity exercise, anaerobic metabolism results in an increase in CHO utilization and in a decrease in NEFA release, uptake and, thus, utilization.

To summarize, during exercise, interactions between CHO and lipid metabolism do not seem reciprocal. While it is established that an increase in CHO utilization results in a decrease in lipid oxidation, the reverse (which would be Randle’s cycle during exercise) is not. But this remains logical as long as we consider the various constraints: at rest, absolute priority is the struggle against hypoglycaemia, even if muscular glucose uptake must be inhibited, whereas during exercise, supplying glucose to the muscle is as much a priority as blood glucose. This is certainly why the body developed an insulin-independent glucose uptake ability in order to spare muscular glycogen. So, in this perspective, the existence of a system inhibiting glucose uptake would be counterproductive. In fact, muscle metabolism seems to privilege lipid more than CHO utilization and plasma more than muscular substrates as far as their speed rate of supplying and oxidation can achieve energy demand.

9. Breaking or bypassing the circles: effects of training

Alteration of exercise and recovery states results in adaptations optimizing energy balance for a given performance. We only discuss here the effects of endurance training, more studied than resistance training, even if there is a current interest in its utilization in diabetes and obesity.
After a description of training effects on substrates metabolism, we will discuss interactions between training status and exercise paradigm. Then, as a conclusion, we will see how training and exercise can interact with regulations loops at rest.

9.1. Training and exercise

Endurance training (ET) results in an increase in maximal power output, also represented by $V_{O_2max}$. ET also increases lipid oxidation and decreases catecholamines production whereas high-intensity exercise causes an increase in CHO utilization and catecholamines concentration [112]. So, interaction between training status and exercise intensity should probably be taken into account to study ET effects on metabolic adaptations during exercise. The crossover concept, as developed by Brooks and Mercier, integrates all those parameters [112]. Glucodependency is defined as the exercise intensity range when CHO become the predominant substrates. According to this concept the crossover point is set as the relative intensity when this shift toward predominant CHO utilization occurs. ET was demonstrated to cause an increase in the crossover point. For example, after a few weeks of ET, exercising at a given relative intensity results in a higher absolute power output and an increased lipid oxidation. The latter is explained by an increase in mitochondrial mass, oxidative enzymatic activity and respiratory channel energy production. In other words, for an identical oxygen flux, trained subjects produce more ATP than untrained subjects. Consequently, this increase in ATP inhibits muscular glycogenolysis, phosphofructokinase, pyruvate-dehydrogenase and, thus, glycolysis which leads to a decrease in lactate production during exercise [113,114].

ET's effects on catecholamines secretion is biphasic. During moderate-intensity exercise, epinephrine is decreased in trained subjects [115] and reaches lipolysis-stimulating concentrations. By contrast, during high-intensity exercise, catecholamine response is increased in trained subjects [116]. It has to be noted that these changes occur early, before circulatory or mitochondrial adaptations, and result in an increase in lipid oxidation for intensities higher than before ET [117].

Pancreas hormones secretions are also modified by ET, which lowers the decrease in insulin secretion and the increase in glucagon secretion. As these changes apparently favour predominant CHO utilization over lipid oxidation, this would seem a paradox if an increase in tissue's sensitivities to those various signals were not taken into account.

ET causes an increase in AT sensitivity to the lipolytic effects of epinephrine during exercise, allowing maintenance of an adapted NEFA flux despite a decrease in epinephrine concentration and an inhibition of decrease in insulin secretion. ET's effects on the liver are discordant among the various studies with an apparent increase in EGP and gluconeogenesis. Considering muscle, ET causes an increase in GLUT-4 pool size and translocation as well as in hexokinase activity [117–120]. IMTG pool size is also increased by ET. As reminded above, a correlation between insulin resistance and IMTG pool size is well established but a causative link is not. This observation led to the debate about the IMTG paradox recently reviewed by Van Loon [104] as this correlation was positive in sedentary, obese and diabetic subjects but negative in healthy trained athletes. This is no longer a paradox if one reasons with oxidative capacity as explained before. More precisely, ET causes an increase in IMTG utilization and, consequently, in NEFA esterification. As NEFA flux is decreased due to ET-induced decrease in catecholamines and in insulin secretion blockade, this increase in IMTG plays a compensating role. A simultaneous ET-induced increase in lipid oxidation, IMTG utilization and NEFA storing could allow the use of IMTG as an alternative substrate to glycogen as it is quickly available with a well-coordinated turnover [104].

Fig. 8. Back from a vicious to a virtuous circle: effect of exercise and training on an adipo-hepato-insulo-muscular loop of regulation modified by an excess of visceral adipose tissue (NEFA: non-esterified fatty acids; VAT: visceral adipose tissue; SCAT: subcutaneous adipose tissue; EGP: endogenous glucose production).
Glycogen metabolism is also a matter of current interest. Glycogen stores are the main factor determining performance and also play a role in regulating substrates’ metabolism. As a matter of fact, when muscular glycogen concentration is low, respiratory exchange ratio (RER) is decreased, lipolysis and lipid oxidation are increased whereas CHO oxidation is unchanged [121]. This suggests for the existence of a signal linking muscular glycogen concentration with lipid metabolism, which is actually unknown. AMPK would be a good candidate but it mainly stimulates CHO metabolism. Other signals, such as IL-6 or TNF-α, are likely to be part of a “work factor” as a signal-modulating metabolism according to glycogen availability more than as a signal of contraction [96,122]. Practically, IL-6 is increased during contraction and its rate of secretion is proportional to glycogen depletion. It contributes to an increase in EGP and a lipolytic activity of this cytokine has also been demonstrated in vitro [122].

10. Conclusion

On the whole, considering that the above-described regulation loops are open, vicious or virtuous circles and that on the other hand exercise and training activate metabolic pathways that are irrelevant at rest, exercise and training appear to be able to compensate mechanisms and thus to turn those circles from vicious to virtuous state.

For example, muscle contraction and training both increase muscular glucose uptake, respectively, in a short-term and a long-term perspective and, most important of all, in an insulin-independent fashion (Fig. 8).

Insulin resistance is defined as a lack of efficiency of a given concentration of insulin to normalize blood glucose. Thus, any mechanism that helps insulin to restore glucose homeostasis decreases insulin resistance. This simple example shows that in order to understand physiology or pathophysiology of metabolic diseases with their apparent paradox and in order to test drug or non-drug treatments of these diseases, vicious and virtuous circles should be taken into account. As long as physiological conditions can be restored, even if the initial pathogenic mechanism is still at work, then acting on these regulation loops could be beneficial. Well-designed studies on the therapeutic use of physical activity, even if suggested since Hippocrates, have been lacking until recently. The recent development of these studies has shown that exercise is able to reverse or delay vicious circles. On the basis of the mechanisms reviewed above, it can be assumed that an individual quantification of these regulation loops will allow targeting optimal exercise parameters aiming at better restoring the virtuous state.

References


