REVIEW ARTICLE

Role of plasma membrane transporters in muscle metabolism

Antonio ZORZANO[1], César FANDOS and Manuel PALACÍN

Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain

Muscle plays a major role in metabolism. Thus it is a major glucose-utilizing tissue in the absorptive state, and changes in muscle insulin-stimulated glucose uptake alter whole-body glucose disposal. In some conditions, muscle preferentially uses lipid substrates, such as fatty acids or ketone bodies. Furthermore, muscle is the main reservoir of amino acids and protein. The activity of many different plasma membrane transporters, such as glucose carriers and transporters of carnitine, creatine and amino acids, play a crucial role in muscle metabolism by catalysing the influx or the efflux of substrates across the cell surface. In some cases, the membrane transport process is subjected to intense regulatory control and may become a potential pharmacological target, as is the case with the glucose transporter GLUT4. The goal of this review is the molecular characterization of muscle membrane transporter proteins, as well as the analysis of their possible regulatory role.

Key words: amino acids, carnitine, creatine, fatty acids, glucose, lactate.

INTRODUCTION

Muscle is subjected to major changes in energy requirements, and as a result it regulates the rate of utilization of various substrates (Figure 1). There is substantial evidence that muscle metabolism relies on the activity of membrane proteins that catalyse the uptake of critical substrates for energy production or for anabolic processes, or the release of glycolytic metabolites or amino acids. There is an internal 'energy store', in the form of phosphocreatine, which is maintained in equilibrium with ATP through catalysis by creatine kinase; therefore any fall in the ATP concentration leads to the formation of further ATP from ADP, using the energy of phosphocreatine. Creatine is the substrate for the synthesis of phosphocreatine, and it is taken up into the muscle cell by creatine carriers (Figure 1). A major substrate under absorptive resting conditions or during exercise is glucose, which is taken up via different glucose transporters that are exquisitely regulated by insulin or exercise. As a result of glucose metabolism, lactate is formed, especially under conditions in which there is an oxygen deficit, and lactate is released via the activity of monocarboxylate transporters. In fact, monocarboxylate transporters are also responsible for the uptake into the muscle cell of ketone bodies (β-hydroxybutyrate or acetoacetate), which are efficient substrates of muscle metabolism. The major lipid substrates in muscle are fatty acids, which are especially important under fasting conditions or during aerobic exercise, and although the mechanism of fatty acid uptake is controversial, there is evidence for the participation of membrane proteins. L-Carnitine is essential for the synthesis of acylcarnitine derivatives, which reach the mitochondrial matrix to undergo β-oxidation; in this regard, carnitine is synthesized mainly in liver and kidney, and it must be taken up by muscle via carnitine transporters found in the plasma membrane.

Muscle is not only an important site for work performance and high energy demand, but it is also the main reservoir of amino acids and protein. This is accomplished by the activity of many different amino acid transporters that carry out the uptake and the release of amino acids. Based on all these considerations, since much has recently been learned about all of these proteins, this review will focus on the membrane proteins that participate in the metabolism of creatine, glucose, amino acids and lipid substrates (such as fatty acids and ketone bodies) in muscle.

CREATINE UPTAKE AND CREATINE TRANSPORTERS IN MUSCLE

Intracellular phosphocreatine is an essential component of energy metabolism in muscles and brain, since it acts as a store of high-energy phosphate which can be converted into ATP by creatine kinase. In turn, phosphocreatine is formed from creatine by creatine kinase. Phosphocreatine is spontaneously converted into creatinine, which is excreted by the kidney; the daily amount of creatinine excreted in the urine is approx. 15 mmol (2 g). This loss of creatine has to be replenished, either from food or by endogenous synthesis. Creatine is synthesized from arginine, glycine and methionine in the liver, pancreas and kidney. Muscles are unable to synthesize creatine, which must therefore be taken up from the bloodstream.

Creatine is increasingly used by athletes as a dietary supplement to improve physical performance when the availability of creatine phosphate is important; indeed, dietary creatine increases muscle phosphocreatine and creatine concentrations in humans [1–3]. Several studies indicate that dietary creatine supplementation enhances performance in high-intensity, short-term exercise or in

Abbreviations used: FABPpm, plasma membrane fatty acid-binding protein; FAT, fatty acid translocase; FATP, fatty acid transport protein; GABA, γ-aminobutyric acid; IGF-I, insulin-like growth factor-I; IRAP, insulin-regulated aminopeptidase; MCT1 (etc.), monocarboxylate transporter 1 (etc.); MEF2, myocyte enhancer factor 2; NSF, *N*-ethylmaleimide-sensitive fusion protein; SCAMP, secretory carrier-associated membrane protein; SNAP-25, 25 kDa synaptosome-associated protein; SNARE, soluble NSF attachment protein receptor; tSNARE, target membrane SNARE; vSNARE, vesicular SNARE; T_3, 3,3′,5-tri-iodothyronine; VAMP, vesicle-associated membrane protein.

[1] To whom correspondence should be addressed (e-mail azorzano@porthos.bio.ub.es).

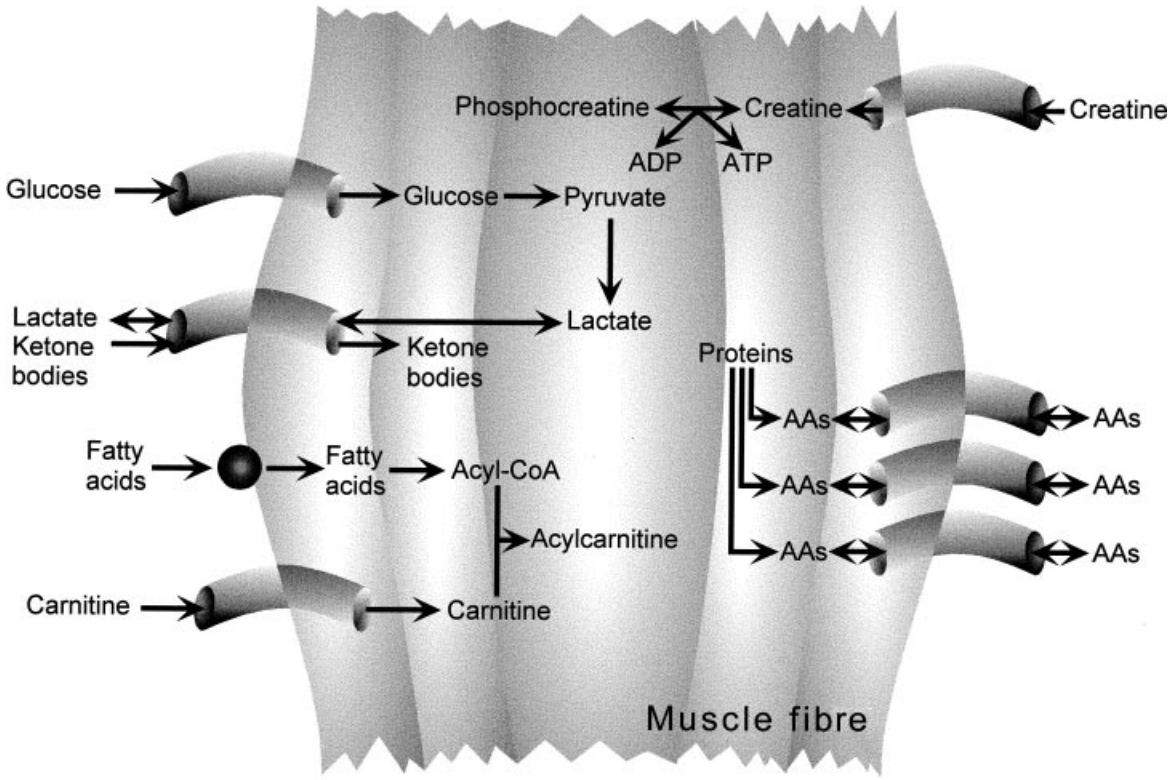

Figure 1 General view of transport of metabolites across the plasma membrane in muscle

AA, amino acid. Lactate and ketone bodies do not indicate the operation of an exchange transport. The multiplicity of amino acid transporters indicates the function of different amino acid transport systems.

intermittent work performance [4,5], although it does not improve sprint performance in swimmers or runners [6,7].

Cardiac and skeletal muscle cells take up creatine by a mechanism that depends on sodium, shows an apparent K_m for creatine in the micromolar range and is inhibited by the structural analogues 3-guanidinopropionate and 4-guanidinobutyrate [8–10]. Creatine uptake in G8 muscle cells is stimulated by long-term exposure to insulin, insulin-like growth factor-I (IGF-I), isoprenaline (isoproterenol) or 3,3′,5-tri-iodothyronine (T_3) [9], probably as a consequence of stimulation of Na^+–K^+-ATPase activity. However, there is some controversy as to the effects of insulin on muscle creatine uptake: whereas some authors have reported stimulation of creatine uptake [11,12], others have found that acute treatment with insulin does not alter creatine uptake by soleus muscle [10]. Creatine uptake by L6 muscle cells is down-regulated in the presence of extracellular creatine [13], and carbohydrate ingestion increases skeletal muscle creatine accumulation during creatine supplementation in humans [14]. All these observations indicate that carnitine uptake is regulated, and that changes in creatine uptake influence the accumulation of creatine by muscle. In consequence, one way to enhance total intracellular creatine in muscle entails the stimulation of creatine transport.

Creatine transport is catalysed in humans by two creatine transporters, named CRT1 and CRT2, which are encoded by different genes. Creatine transporters belong to the superfamily of Na^+- and Cl^--dependent neurotransmitter transporters [15]; in keeping with this, expression of CRTs in *Xenopus* oocytes or in COS-7 cells induces the uptake of creatine, which is dependent on Na^+ and Cl^- ions [16,17]. The stoichiometry of creatine, Na^+ and Cl^- seems to be 1:2:1 [17]. The creatine transporters present twelve transmembrane domains, and human CRT1 shows two potential N-glycosylation sites (Figure 2a) [18]. Although the specific structure/function relationship of the creatine transporters has not been studied, they show the greatest identity with γ-aminobutyric acid (GABA) and taurine transporters. In humans, cardiac muscle and skeletal muscle, together with brain, kidney and placenta, express CRT1 [18]. To date, the cDNAs for human, rat, rabbit and *Torpedo* CRT1 creatine transporters have been identified [16,18–21], and functional analysis indicates that they encode a creatine transporter which shows a K_m for creatine in the micromolar range, i.e. very similar to the kinetic data obtained in muscle cells [14]. The human *CRT1* gene is located on chromosome Xq28, it contains 13 exons and spans approx. 8.5 kb of genomic DNA [21,22]. In contrast with the broad expression of CRT1, human CRT2 is only expressed in testis, and is located on chromosome 16 (16p11.2) [23,24].

There are many unanswered questions with regard to the biology of muscle creatine transporters, such as the precise mechanism of transport, structure/function relationships, and regulation of the subcellular distribution and activity of the carrier. It has been reported that creatine supplementation of rats down-regulates the expression of CRT1 in skeletal muscle [25], which is in agreement with the above-mentioned observation that extracellular creatine down-regulates creatine uptake [13].

GLUCOSE TRANSPORTERS REGULATE GLUCOSE UTILIZATION BY MUSCLE

Skeletal muscle accounts for nearly 40 % of body mass, and is the main tissue involved in the insulin-induced stimulation of glucose

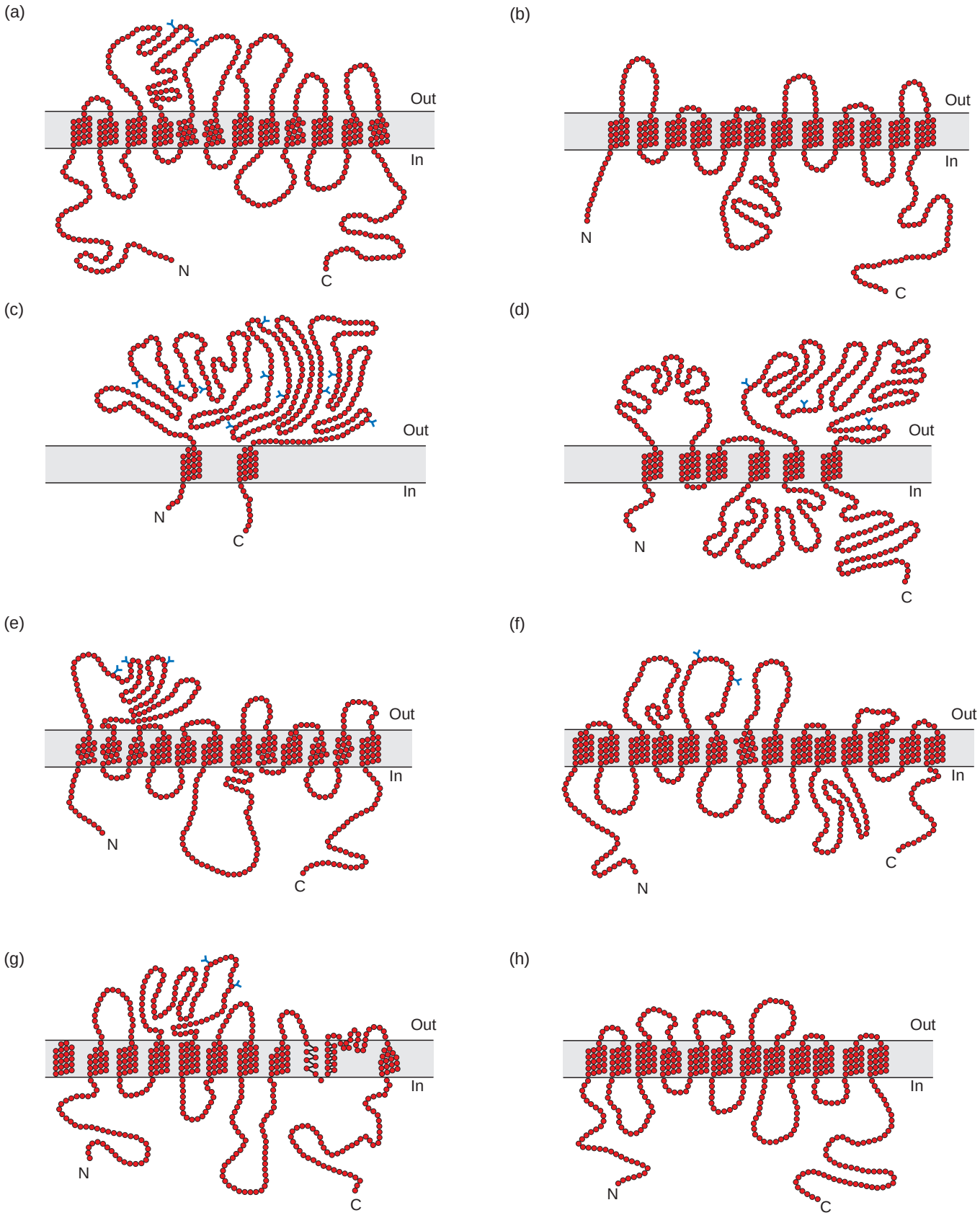

Figure 2 Proposed topology for different membrane transporters expressed in muscle

(**a**) Human creatine transporter (CRT1); (**b**) *Cricetulus longicaudatus* (Chinese hamster) MCT1; (**c**) rat FAT/CD36; (**d**) mouse FATP-1; (**e**) human carnitine transporter OCTN2; (**f**) human CAT-1 transporter; (**g**) human anionic amino acid transporter EAAT1; (**h**) human L-amino acid transporter LAT-2. Putative glycosylation sites are indicated by blue branches.

uptake. Several studies using the euglycaemic–hyperinsulinaemic clamp have shown that, at circulating levels of insulin in the upper physiological range, most of the infused glucose is taken up by skeletal muscle and converted mainly into glycogen [26]. Evidence for the role of muscle glucose uptake in overall glucose homoeostasis also comes from studies in transgenic mice overexpressing the GLUT1 glucose transporter in skeletal muscle. Thus overexpression of GLUT1 in transgenic mice increases glucose uptake into muscle, which leads to low plasma glucose concentrations and an increased rate of glucose disappearance after a glucose tolerance test [27]. Because of its quantitative role in glucose uptake, alterations in the insulin sensitivity of muscle have a profound impact on whole-body glucose disposal. In this regard, patients with Type-II (non-insulin-dependent) diabetes show deficient insulin-induced glucose transport in skeletal muscle [28,29].

Insulin treatment, exercise or electrical stimulation rapidly increase the rate at which glucose is taken up by muscle. Kinetic analysis of the effects of insulin or contraction on muscle glucose transport indicate an increase in the V_{max} value [30]. The stimulation of muscle glucose transport is critical, since this process is thought to be a rate-limiting step in the pathway of glucose utilization in skeletal muscle. Several observations support this view: (a) intracellular free glucose does not accumulate, regardless of the glucose or insulin concentration, in skeletal muscle from control or streptozotocin-induced diabetic rats [31,32], (b) in normoglycaemic conditions, both in the absence of insulin and at submaximal insulin concentrations, glucose clearance is constant in the perfused rat hindlimb [33], and (c) overexpression of GLUT1 in skeletal muscle from transgenic mice leads to a 10-fold increase in muscle glycogen content and a 2-fold increase in muscle lactate, with no increase in the muscle concentration of glucose 6-phosphate [34].

Different glucose transporters are expressed in muscle

Skeletal muscle expresses GLUT1 and GLUT4 glucose transporters [35]. Besides GLUT4 and GLUT1, there is some evidence that the skeletal muscle of humans, but not that of rats, expresses GLUT5 [36,37]. Based on the induction of fructose transport activity in *Xenopus* oocytes after injection of human GLUT5 cRNA [38], it is thought that GLUT5 accounts for fructose uptake by muscle. It has been reported recently that skeletal muscle also expresses low levels of mRNA for GLUTX1, a novel glucose transporter with unknown physiological properties [39].

GLUT4 is the main glucose carrier expressed in skeletal muscle from adult rats, whereas GLUT1 accounts for only 5–10 % of total glucose carriers [40], which is similar to data reported for isolated rat adipocytes [41]. The structure/function relationships of these 12-transmembrane-domain proteins, which have both the N- and C-terminal domains oriented to the cytosol, have recently been reviewed elsewhere [42], and they will not be analysed here.

Cardiac muscle also expresses both GLUT4 and GLUT1 glucose transporters; in rat cardiomyocytes, these account for approx 60 % and 40 % respectively of total glucose carriers [43].

In addition to the differences in their expression levels, GLUT1 and GLUT4 show a differential localization in the muscle fibre. Results obtained from subcellular fractionation studies or by immunoelectron microscopy indicate that GLUT1 carriers are located mainly in the plasma membrane, whereas GLUT4 carriers are more abundant in intracellular membranes (for a review, see [35]).

The expression of muscle GLUT4 is regulated

The relative abundances of GLUT4 and GLUT1 in skeletal and cardiac muscles depend on the developmental stage [44]. Thus, during fetal life in the rat, GLUT1 is the predominant glucose carrier, and its expression is markedly repressed perinatally [44–46], as a consequence of alterations occurring at a pretranslational stage [44,45]. In contrast, muscle expression of GLUT4 is low in the fetal rat, and a continuous induction of GLUT4 mRNA and protein takes place in the perinatal phase [44–46]. The expression of glucose transporter isoforms during perinatal life is consistent with the observation of a high rate of glucose uptake by fetal rat heart [47].

With regard to the mechanisms responsible for the transitions in muscle GLUT1 and GLUT4 expression during the perinatal phase, thyroid hormones play an important role. This view is based on the observation that congenital hypothyroidism markedly impairs GLUT4 protein induction when GLUT4 mRNA levels are low. The effect of the deficit in thyroid hormones on GLUT4 expression is reversible, and a single injection of T_3 causes a marked and rapid increase in the levels of GLUT4 mRNA and protein in the heart [46]. Similarly, congenital hypothyroidism leads to a substantial enhancement of GLUT1 protein and mRNA levels in the heart [46]. Furthermore, injection of hypothyroid neonates with T_3 causes a decrease in the cardiac levels of GLUT1 mRNA [46]. Thyroid hormones are also involved in the control of glucose transport in skeletal muscle in adulthood. Thus chronic T_3 administration stimulates GLUT4 expression and glucose transport in skeletal muscle from rats [48,49].

A role for thyroid hormones in controlling the transition of glucose transporters from fetal to neonatal levels in heart is also supported by additional data. Thus there is analogous behaviour between circulating concentrations of thyroid hormone and the induction of cardiac GLUT4 protein during perinatal development: thyroxine (T_4) and T_3 levels increase progressively during postnatal life and reach a plateau 2 weeks after birth [50]; in parallel, cardiac GLUT4 protein expression increases after birth, becomes progressively more abundant, and attains adult levels after day 15 of postnatal life [44,46].

The contractile activity that is dependent on innervation regulates the expression of GLUT1 and GLUT4 in skeletal muscle in an inverse manner. This is supported by two distinct lines of evidence: (a) the induction of GLUT4 and the repression of GLUT1 occur at the end of fetal life and coincide with the timing of skeletal muscle innervation [51], and (b) the expression of GLUT1 is enhanced and that of GLUT4 is repressed in response to muscle denervation during adult life [51–53].

In addition, chronic low-frequency stimulation of muscle *in vivo* or *in vitro* causes the induction of GLUT4 expression above basal levels [54–56]. Physical training increases insulin-mediated whole-body glucose utilization in human subjects, which reflects an adaptation in muscle elicited by local contraction-dependent mechanisms. Physical training in humans does not alter muscle insulin receptor function, but it enhances the muscle content of GLUT4 [57,58]. Similarly, chronic exercise enhances GLUT4 expression in muscle from rats [59]. In fact, it seems that a single bout of exercise enhances GLUT4 expression in muscle [60]. Furthermore, chronic exercise also increases GLUT4 content in skeletal muscle from obese insulin-resistant Zucker (fa/fa) rats [61].

In spite of its potential therapeutic importance, there is relatively little information on the specific mechanisms that regulate GLUT4 gene transcription in muscle. Studies performed in transgenic mice have shown that 2.4 kb of the 5′-flanking

region of the human GLUT4 gene fused to a chloramphenicol acetyltransferase reporter gene is specifically expressed in adipose tissue, skeletal muscle and heart [62], which indicates that this 2.4 kb of the GLUT4 gene contains all the sequence elements needed to confer tissue-specific expression.

Work using transgenic mice has also shown skeletal-muscle-specific DNA elements located within 730 bp of the GLUT4 5′-flanking DNA [63]. These results are consistent with other studies in C_2C_{12} muscle cells in culture which have shown that muscle-specific expression of GLUT4 is conferred by a 103 bp DNA sequence located between positions −522 and −420 of the rat GLUT4 gene [64]. A myocyte enhancer factor 2 (MEF2) binding site in the GLUT4 promoter located between positions −466 and −457 relative to the transcription start site has also been proposed as being essential for the specific expression of GLUT4 in muscle cells [64].

Studies performed in C_2C_{12} muscle cells have shown that thyroid hormones stimulate the transcription of the rat GLUT4 gene through a 281 bp region in the GLUT4 promoter located between positions −517 and −237 relative to the transcription start site [65]. Torrance et al. [66] have proposed a new, low-affinity binding site for thyroid hormone receptors in the GLUT4 promoter, located between bases −457 and −426, next to the MEF2 site, although the functional role of this site has not been demonstrated. These results are consistent with reports that thyroid hormones stimulate GLUT4 gene expression in muscle [46].

It has also been reported that 2400 bp of 5′-flanking DNA is sufficient for regulation of the human GLUT4 gene in transgenic mice during fasting and refeeding [62]. Furthermore, a 1154 bp fragment is necessary to direct the insulin-dependent regulation of the human GLUT4 gene in muscle and adipose tissue from transgenic mice [63].

At present, there are several indications that the regulation of GLUT4 expression in muscle *in vivo* is due to modifications not only at a transcriptional level but also post-transcriptionally. Thus the amount of GLUT4 protein decreases in the presence of slight or even no changes in GLUT4 mRNA levels in red skeletal muscle from 2-day-fasted rats or in white muscle from rats with streptozotocin-induced diabetes [67]; similarly, the amount of GLUT4 protein decreases in the absence of changes in its mRNA in skeletal muscle from benfluorex-treated rats [68]. GLUT4 protein has also been reported to increase in the absence of changes in mRNA levels in rat heart during perinatal development [44] and in skeletal muscle from the mdx dystrophic mouse [69].

On the other hand, alterations in tissue levels of GLUT4 mRNA have been detected in the absence of changes or in the presence of inverse changes in the content of GLUT4 protein. Thus, in hearts from propylthiouracil- or methylmazole-induced hypothyroid rats, GLUT4 protein does not change when the amount of GLUT4 mRNA diminishes markedly [46,70]. In skeletal muscle from rats with glucose-induced hyperglycaemia [71], GLUT4 mRNA increases without affecting GLUT4 protein levels. Similarly, streptozotocin-induced diabetes causes a large decrease in cardiac GLUT4 mRNA levels which is, however, accompanied by only a small decrease in cardiac GLUT4 protein [67]. The precise mechanisms that regulate this adaptation – changes in translational efficiency or in the rate of GLUT4 protein degradation – are unknown. However, it has been reported that treatment of streptozotocin-induced diabetic rats with ICI D8731 – an antagonist of the angiotensin type-1 receptor – for 4 months prevents the decrease in cardiac GLUT4 protein under conditions in which mRNA levels stay low [72]; this suggests that angiotensin II influences, either directly or indirectly, the post-transcriptional regulation of GLUT4 expression in the heart.

All of these observations suggest that the regulation of muscle GLUT4 expression at translational or post-translational steps is relatively frequent, and can involve either the preservation of GLUT4 carrier levels in the presence of variable changes in GLUT4 gene expression or the modification of GLUT4 carriers in the absence of changes in GLUT4 mRNA. These two inverse patterns can be explained by changes in the efficiency of translation of GLUT4 transcripts or in the general machinery of protein synthesis, or by modifications in the rate of GLUT4 protein degradation. In this regard, we have no knowledge of the specific regulatory mechanisms that occur at the translational and/or post-translational levels. Much effort is required to unravel the molecular basis for the control of GLUT4 expression at the post-transcriptional level.

Insulin and muscle contraction cause translocation of GLUT4 to distinct cell surfaces in muscle

As mentioned above, glucose transport in skeletal muscle and cardiac muscle is thought to be maintained by the catalytic activity of two glucose transporter isoforms, i.e. GLUT4 and GLUT1. In non-stimulated conditions GLUT1 is found mainly in the sarcolemma of the muscle fibre, but not in transverse tubules [40,73]. In contrast, GLUT4 is mostly associated with intracellular membranes [35]. Intracellular GLUT4 is found in large elements, including multivesicular endosomes located in the trans-Golgi network region, and in small tubulo-vesicular structures [74]. Analysis of subcellular distribution in transgenic mice overexpressing GLUT4 by immunogold labelling has revealed that most GLUT4 is located in and around the muscle triad [75]. A variety of experimental approaches have shown that, in non-stimulated conditions, GLUT4 is mainly intracellular in cardiac myocytes [43,76]. Immunoelectron microscopy of rat heart has shown that GLUT4 labelling is mostly associated with intracellular membranous vesicular structures [77]. These tubulo-vesicular elements are found in different locations: (a) perinuclear elements near the Golgi apparatus, (b) near the sarcolemma, (c) near the transverse tubule membranes and (d) near the intercalated disks [78].

Immunocytochemical studies, subcellular fractionation and photolabelling assays (reviewed in [35]) indicate that GLUT4 is translocated from an intracellular locus to the cell surface of the muscle fibre in response to insulin or exercise. GLUT4 is recruited to selective domains of the sarcolemma [73] and to T-tubules of the muscle fibre [74,79,80] (Figure 3). There is controversy as to whether insulin unmasks a C-terminal GLUT4 epitope in T-tubules in skeletal muscle [74,75].

There is also evidence that insulin promotes a marked recruitment of GLUT4 carriers to the cell surface in cardiac myocytes [77]. Indeed, GLUT4 is recruited in response to the combination of insulin and exercise both to the sarcolemma and to the T-tubule of cardiac myocytes [77]. Insulin also redistributes GLUT1 from an intracellular site to the cell surface in isolated rat cardiomyocytes or in perfused rat heart [43,76,81]. Nevertheless, the effect of insulin on GLUT1 is clearly weaker than its effect on GLUT4 [43,76]. The insulin-dependent recruitment of GLUT1 in cardiomyocytes contrasts with the lack of such an effect in skeletal muscle, but is similar to the effect observed in the adipose cell, in which both GLUT4 and GLUT1 translocate in response to insulin [41].

Exercise and muscle contraction also stimulate glucose transport and cause translocation of GLUT4 to the cell surface in

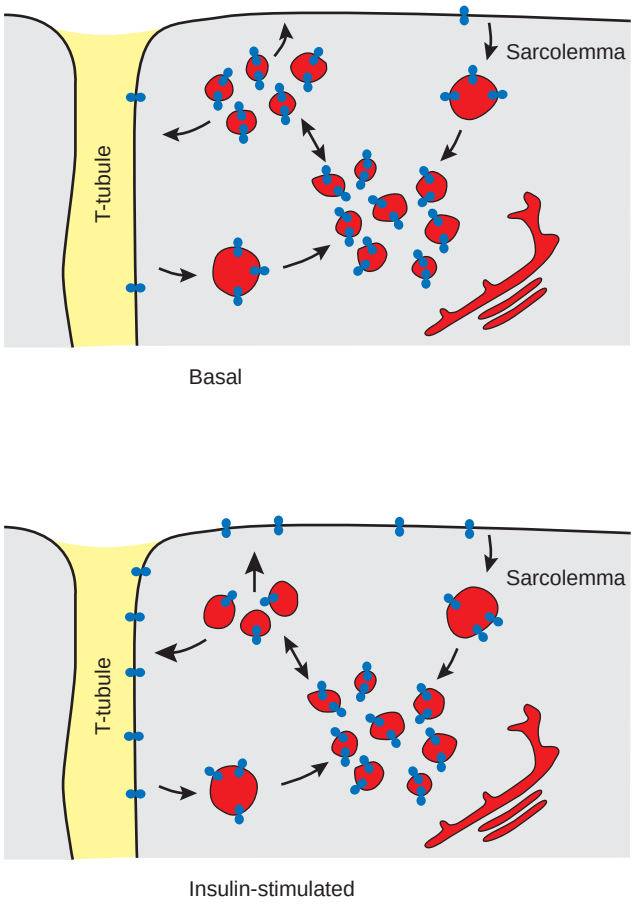

Figure 3 Scheme of the hypothetical translocation of GLUT4 in the muscle fibre in response to insulin

Insulin receptors are present both in sarcolemma and in T-tubules, and insulin promotes the translocation of GLUT4 from intracellular compartments to the sarcolemma and T-tubules. Whether the intracellular GLUT4 vesicles move in a non-distinct manner to sarcolemma and T-tubules, or whether there is specialization, is unknown.

skeletal muscle [82,83]; the recruitment of GLUT4 triggered by exercise in rat skeletal muscle affects both sarcolemma and T-tubules [74]. GLUT4 is also translocated to the cell surface in response to contraction, hypoxia or ischaemia in cardiac myocytes [84,85].

In summary, a distinctive feature of muscle cells is that two distinct cell surfaces, i.e. sarcolemma and T-tubules, are involved in recruitment of GLUT4 in response to insulin or exercise. Based on this, it is likely that there are important differences in GLUT4 traffic between adipose and muscle cells [86].

GLUT4 trafficking pathway in muscle

GLUT4 trafficking involves several steps in both cardiac muscle and skeletal muscle. Thus an intracellular exercise-sensitive GLUT4 pool has been identified by subcellular fractionation of rat skeletal muscle; this GLUT4 pool shows no sensitivity to insulin [87], which indicates that distinct pools may be responsible for exercise- and insulin-dependent GLUT4 translocation.

Additional evidence has come from the purification of two intracellular GLUT4 membrane pools from rat skeletal muscle that differ with respect to their response to insulin *in vivo*: one shows a marked decrease in GLUT4 levels, while the other is unaltered [80,88–91]. These two GLUT4 membrane populations also differ in their polypeptide composition. Vesicle immunoisolation analysis performed with insulin-sensitive or insulin-insensitive intracellular membrane fractions has revealed that SCAMPs (secretory carrier-associated membrane proteins), cellubrevin and transferrin receptors are only present in the insulin-insensitive GLUT4 pool. In contrast, VAMP2 (vesicle-associated membrane protein 2) and IRAP (insulin-regulated aminopeptidase) are detected in GLUT4 vesicles isolated from both intracellular membrane fractions [90,91].

There is also evidence that there are at least two intracellular GLUT4 vesicle populations in rat cardiomyocytes [43]. Thus GLUT4 vesicles were immuno-isolated using various amounts of anti-GLUT4 antibody from intracellular membranes from non-stimulated cardiac myocytes [43]. About 40–50 % of the GLUT4 vesicles present in intracellular membranes from cardiomyocytes were immuno-isolated using small amounts of anti-GLUT4 antibody; under these conditions, very little GLUT1 or SCAMP was detected in the immunoprecipitates. Larger amounts of antibody caused maximal immunoadsorption of GLUT4-containing vesicles, and under these conditions most of the GLUT1 and SCAMP was detected in the immunoprecipitates [43].

Analysis of the distribution of GLUT4 in skeletal muscle fibres has also identified distinct intracellular GLUT4 compartments. Thus, in non-stimulated conditions, approx. 23 % of intracellular GLUT4 is associated with large structures located in the trans-Golgi network region, and 77 % with small tubulo-vesicular structures [74]; both compartments are recruited by insulin and contraction [74]. In turn, the GLUT4 detected in small tubulo-vesicular structures can be further subdivided into transferrin-receptor-positive and transferrin-receptor-negative elements [74].

The observations in skeletal muscle and in cardiac myocytes are consistent with the presence of at least two intracellular GLUT4 membrane populations in non-stimulated muscle cells (see Figure 4): (a) an endosomal GLUT4 compartment with a high content of GLUT1 and SCAMPs in cardiac myocytes, and a high abundance of SCAMPs, transferrin receptors and cellubrevin in skeletal muscle; and (b) storage/exocytic GLUT4 vesicles with a low content of GLUT1 and SCAMPs in cardiomyocytes, and a high content of VAMP2 and IRAP in skeletal muscle. The pattern of protein composition of these two GLUT4 pools suggests that the storage pool derives from the endosomal pool. According to this proposal, GLUT4 would be sorted from the endosomal membrane population to the storage/exocytic pool together with VAMP2 or IRAP (at least in skeletal muscle), and the storage compartment would be subjected to recruitment in response to insulin (Figure 4). In cardiac myocytes, there is also evidence that insulin recruits the endosomal compartment to the cell surface [43]. However, direct evidence that GLUT4 moves from the cell surface to the endosomal population, that GLUT4 is sorted from the endosomal to the storage pool or that exocytic GLUT4 vesicles are recruited directly to the cell surface is lacking.

Putative proteins involved in glucose transporter trafficking in muscle

As reviewed above, there is evidence that GLUT4 moves through a series of steps in muscle cells and, therefore, that different proteins control the various trafficking processes. Our current knowledge on this issue is scarce, and most of the work has been done in adipose cells or in heterologous cell systems.

Non-metabolizable analogues of GTP stimulate recruitment of GLUT4 to the cell surface of permeabilized adipocytes and

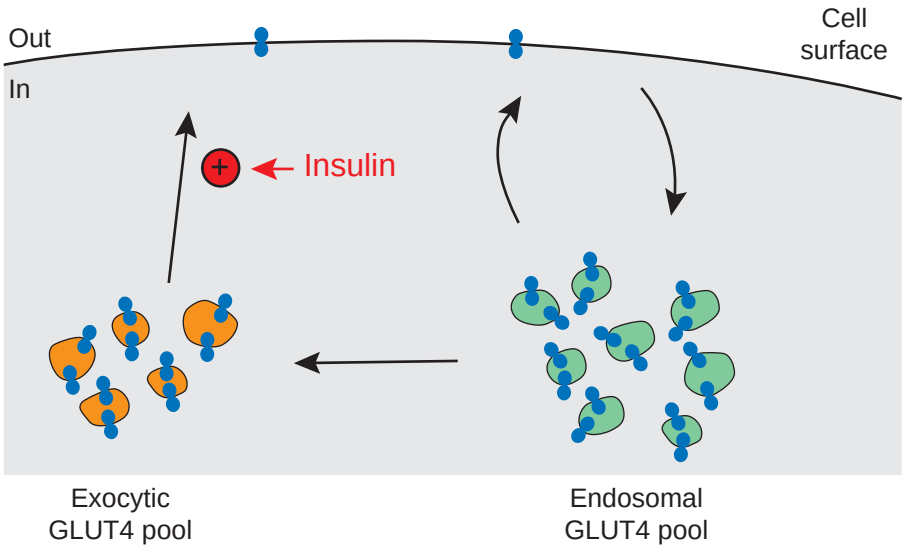

Figure 4 Scheme of proposed GLUT4 trafficking pathway in skeletal muscle and cardiac myocytes

Results obtained in skeletal muscle and cardiac myocytes are consistent with GLUT4 being present in an endosomal compartment (which selectively contains cellubrevin and SCAMPs in skeletal muscle, and SCAMPs and GLUT1 in cardiac myocytes) and in an exocytic/storage compartment, which contains a more limited number of proteins. This latter compartment is recruited to the cell surface in response to insulin. The endosomal compartment comes from internalization of GLUT4. There is evidence, although not shown here, that the endosomal GLUT4 compartment in isolated rat cardiac myocytes undergoes recruitment to the cell surface in response to insulin [43].

cardiac myocytes [92,93]. Based on this, several authors have searched for the presence of a GTP-binding protein. rab4 (a small GTP-binding protein) was detected in GLUT4 vesicles derived from adipocytes and from skeletal muscle [94,95]. Interestingly, rab4 is found in early endosomes in CHO cells, it is excluded from the recycling compartment [96,97] and its overexpression causes a redistribution of transferrin receptors from endosomes to the plasma membrane [98]. The overexpression of rab4 in adipocytes also increases the intracellular retention of GLUT4 [99]. Similarly, co-expression of rab4 and GLUT4 in *Xenopus* oocytes reduces the abundance of GLUT4 at the cell surface and diminishes glucose transport [100]. Furthermore, transfer of a synthetic peptide corresponding to the C-terminal domain of rab4 to adipose cells inhibits insulin-induced GLUT4 translocation [101]. Insulin, but not exercise, caused a decrease in rab4 levels in intracellular membranes from rat skeletal muscle [95]. All these results support the view that rab4 indeed regulates GLUT4 trafficking in adipose and muscle cells. Based on all these observations, it is likely that rab4 promotes enhanced sorting of GLUT4 from the endosomal to the storage compartment.

However, rab4 is not the only small GTP-binding protein involved in GLUT4 trafficking in muscle cells. Thus a low-molecular-mass GTP-binding protein, p24, has been detected in GLUT4 vesicles obtained from rat heart [102], and insulin causes a marked decrease in the abundance of p24 associated with intracellular GLUT4 vesicles [102]. The identification of this protein and the analysis of its role in GLUT4 traffic remain to be achieved.

Vesicle fusion in eukaryotic cells is regulated by tSNARE [target membrane soluble NSF (*N*-ethylmaleimide-sensitive fusion protein) attachment protein receptor] and vSNARE (vesicular SNARE) proteins. In this regard, proteins originally identified as tSNAREs involved in synaptic vesicle exocytosis, such as syntaxin 1A/1B or syntaxin 4, have also been found in cell surface membranes from isolated rat adipocytes, cardiomyocytes and skeletal muscle [91,103]. Furthermore, the vSNARE proteins VAMP2 and cellubrevin have been detected in GLUT4 vesicles derived from rat skeletal muscle [91], and cellubrevin has also been found in GLUT4 vesicles derived from isolated rat cardiomyocytes [91]. These results are consistent with previous reports indicating that cellubrevin and VAMP2 co-localize with GLUT4 in intracellular membranes obtained from adipocytes [104,105]. With regard to the role of these proteins in GLUT4 trafficking, it has been reported that the cleavage of VAMP2 and cellubrevin by botulinum neurotoxin B or D inhibits the translocation of GLUT4 in 3T3-L1 adipocytes [106,107]. In addition, introduction of the cytoplasmic domain of syntaxin 4, VAMP2 or cellubrevin results in inhibition of insulin-stimulated GLUT4 translocation to the plasma membrane in 3T3-L1 adipocytes [108]. In summary, the presence of VAMP2 in the exocytic GLUT4 compartment suggests that it plays a role in the docking and fusion of GLUT4 vesicles to the cell surface in the muscle fibre. Cellubrevin interacts with syntaxin 1 and SNAP-25 (25 kDa synaptosome-associated protein) [109], and seems to be involved in the exocytosis of transferrin-receptor-containing vesicles in CHO cells [110], but it does not affect the fusion of early endosomes *in vitro* [111]. Based on the localization of cellubrevin in the endosomal GLUT4 compartment, this protein probably has a role in the fusion events involved in the sorting of GLUT4 from the endosomal to the exocytic pool.

There may also be differences in the specific vSNAREs and tSNAREs responsible for the traffic of GLUT4 in insulin-sensitive cells. Thus cardiac myocytes express syntaxin 1A/1B as well as SNAP-25, and these proteins are mainly located at the plasma membrane [91]. In contrast, adipocytes do not express either syntaxin 1 or SNAP-25, although they do express SNAP-23 [112,113]. In addition, cardiac myocytes do not express VAMP2, which rules out a role for this protein in GLUT4 exocytosis, in contrast with what occurs in adipose cells and skeletal muscle, where VAMP2 co-localizes with GLUT4 in the storage compartment [91,105]. Whether these differences are

attributable to the operation of distinct isoforms or to the acquisition of vSNARE proteins during exocytosis is unknown.

Semicarbazide-sensitive amine oxidase has been found to co-localize with GLUT4 in the endosomal compartment from adipose or muscle cells [114,115]. Interestingly, substrates of semicarbazide-sensitive amine oxidase in combination with very low concentrations of vanadate stimulate glucose transport and translocation of GLUT4 to the cell surface in isolated rat adipocytes [115,116]. Whether these proteins have a regulatory role in GLUT4 trafficking remains unanswered.

Another protein that participates in insulin-induced GLUT4 translocation in cells is protein kinase B, also called akt, which lies downstream from phosphatidylinositol 3-kinase. In fact, insulin, but not muscle contraction, activates akt in rat skeletal muscle [117]. akt-2 has been found in GLUT4 vesicles obtained from isolated rat adipocytes treated previously with insulin [118]. Furthermore, akt-2 phosphorylates proteins that are components of GLUT4 vesicles in response to insulin [119]. This strongly supports the concept that akt-2 phosphorylates a component responsible for triggering the exocytosis of GLUT4 to the cell surface, and it might serve to link insulin signalling to the activation of GLUT4 exocytosis. No data are yet available concerning the association of akt-2 and GLUT4 vesicles in muscle.

MONOCARBOXYLATE TRANSPORTERS MEDIATE LACTATE RELEASE, AS WELL AS UPTAKE OF LACTATE AND KETONE BODIES, BY MUSCLE

Metabolism of monocarboxylic acids in muscle

Skeletal muscle produces and removes lactate at the same time. Lactate is formed during periods of high energy demand or during rapid fluctuations in energy requirements. The formation of lactate during exercise is a result of an excess of pyruvate formation and is not necessarily the result of anaerobic conditions, since lactate can be produced in oxidative fibres of fully oxygenated muscles. The lactate taken up by the muscle can be used for glycogen synthesis, especially in fast-twitch fibres [120], or for oxidation in slow-twitch fibres [121,122]. Thus, at any given moment, lactate can be produced in glycolytic fibres and oxidized in more oxidative fibres. Due to its large mass, skeletal muscle is the main producer and consumer of lactate in the body. Other monocarboxylate compounds are ketone bodies and 2-oxo acids derived from amino acids. Ketone bodies are actively consumed by muscle during fasting or during recovery after exercise, when concentrations of β-hydroxybutyrate or acetoacetate in plasma are high. In contrast, it has been reported that a substantial portion of the branched-chain amino acids deaminated by muscle are released into the circulation as 2-oxo acids [123,124].

Lactate uptake and release are stereospecific, inhibitable by cinnamate and pH-dependent [125]. Lactate uptake by soleus muscle shows a K_m of approx. 13 mM [126]. Lactate flux across the membrane regulates intracellular muscle pH. Thus measurements of intracellular pH after muscle contraction have demonstrated that pH recovery is inhibited by the lactate transport inhibitor cinnamate [127]. Studies using sarcolemmal vesicles have revealed that lactate transport is transactivated by lactate and that a high H^+ concentration at the trans side inhibits the transport [128]. It is also clear that lactate transport is stimulated by protons at the cis side, which has been attributed to fast H^+ binding to the carrier [125]. The transport kinetic parameters and the observed substrate selectivity have led to the concept of lactate–proton co-transport in skeletal muscle [129] in which the transport is stereospecific and electroneutral, probably with a stoichiometry of 1:1, with H^+ binding first to the carrier.

Obviously, efflux of lactate and H^+ from muscle is important during exercise, since it lowers the intracellular concentration of both species and prevents cellular acidification. Some indirect observations support the view that lactate transport controls lactate utilization in skeletal muscle; thus under many different experimental conditions there is a considerable inward-directed lactate gradient [130–132], which suggests that the capacity for lactate transport across the membrane is not much higher than the internal utilization. This remains to be established.

Lactate transport is not subjected to acute regulation in muscle. However, there are several examples indicating that lactate transport is regulated in a long-term manner. Thus lactate uptake is faster in soleus than in extensor digitorum longus muscle in rats [133]; similarly, lactate transport is greater in sarcolemmal vesicles derived from slow-twitch fibres than in vesicles from fast-twitch fibres [134]. These data suggest a higher lactate–proton transport rate in red than in white fibres. Furthermore, situations characterized by up-regulation of lactate transport in skeletal muscle include exercise training [135,136], chronic electrical stimulation [137] and administration of thyroid hormones [138]. In contrast, other conditions such as muscle denervation [139,140], unweighting in rats [141] or aging [134] down-regulate lactate transport.

Monocarboxylate transporters

Different membrane transporter proteins catalyse the proton-linked transport of lactate, pyruvate, branched-chain 2-oxo acids derived from leucine, valine and isoleucine, β-hydroxypyruvate, acetoacetate and acetate, and these have been termed monocarboxylate transporters. At present, eight different monocarboxylate transporter isoforms have been identified in human tissues (MCT1–MCT8; the last is also known as XPCT). An extensive review of monocarboxylate transporters by Halestrap and Price has recently been published [142].

The first monocarboxylate transporter identified, MCT1, was isolated from CHO cells [143]. MCT1 isoforms from human, rat and mouse have now been cloned and share approx. 95% sequence identity with Chinese-hamster MCT1 [144–148]. Human MCT1 shares high identity with human MCT2, MCT3 and MCT4 (ranging from 45 to 57%) and lower identity with the rest of the members so far identified (25–30% identity) [143,149].

Hydrophobicity analysis of the monocarboxylate transporters suggests a structure with 12 transmembrane segments, presumed to be α-helical. It seems probable that there are 12 transmembrane domains, with the N- and C-termini oriented to the cytosol. Furthermore, the MCT family members show the highest sequence conservation in the putative transmembrane region. Results obtained in studies of proteolytic digestion [150] are in keeping with a model of MCT1 like that presented in Figure 2(b). In addition, MCT1 does not show N-glycosylation [146], which raises the question of the nature of the mechanisms involved in intracellular trafficking. In this connection, there is a possible association of MCT proteins and an ancillary protein OX-47, which may be instrumental in directing MCTs to the cell surface (for a review, see [142]). An association between a 12-transmembrane-spanning domain protein transporter and an ancillary protein has been well substantiated for some amino acid transporters (see below).

The transport characteristics of MCT1 have been studied extensively in human erythrocytes (which only express MCT1) and after heterologous expression in *Xenopus* oocytes [151–154]. Kinetics studies indicate that MCT1 is a proton–lactate co-transporter that follows an ordered, sequential mechanism

[151,152]. Transport begins with proton binding to the transporter, which is followed by binding of lactate anion. Translocation of lactate and proton across the membrane occurs next, followed by their sequential release on the other side of the membrane. MCT1 can transport a wide variety of short-chain monocarboxylates, with the K_m value decreasing as the chain length increases from two to four carbon atoms. Monocarboxylates substituted in the C-2 and C-3 positions are good substrates, with the carbonyl group being especially favoured, and C-2 substitution is preferred over C-3. Transport of lactate is stereospecific, but that of other substrates, such as β-hydroxybutyrate or 2-chloropropionate, is not. Monocarboxylates with longer branched aliphatic or aromatic side chains also bind to the transporter, but are not quickly released following translocation and may act as potent inhibitors. MCT4 has a lower affinity for lactate ($K_m > 20$ mM) than does MCT1 (K_m 0.5 mM) after heterologous expression in *Xenopus* oocytes [155]. No functional data are available for MCT5, MCT6, MCT7 or XPCT.

Skeletal muscle expresses MCT1 and MCT4 monocarboxylate transporters [143,155–157]. MCT1 expression correlates with its mitochondrial content. Thus rat muscles enriched in slow oxidative fibres express large amounts of MCT1, whereas muscles with a high content of fast-twitch glycolytic fibres contain hardly any [155,156]. Similarly, there is a positive correlation between MCT1 expression and the occurrence of type-I fibres in human muscles [158]. In contrast, MCT4 is present in all rat muscles, but to a lesser extent in predominantly oxidative muscle [156], and the density of MCT4 is independent of fibre type in human muscle [158]. Heart expresses high levels of MCT1 in both human and rat [142,143]; MCT1 is found at the cell surface of cardiomyocytes, and is particularly concentrated in the intercalated discs [142,143]. MCT1 is not the only monocarboxylate transporter that is expressed in heart, although there is some variability among species; thus human, but not rat, heart expresses MCT4 [143], and Syrian hamster heart expresses MCT2 [159]. Furthermore, Northern blot data suggest that both human skeletal muscle and heart express other monocarboxylate transporters with as yet unknown functions: skeletal muscle expresses MCT6 and XPCT, and heart expresses MCT5, MCT6, MCT7 and XPCT [149]. In all, data so far available indicate the expression of different MCT isoforms in skeletal muscle and cardiac muscle, and further studies are required to characterize specific cellular distributions. Based on the distribution of MCT1 and MCT4 in different muscle fibres, it has been proposed that MCT1 expression in skeletal muscle may reflect the need to take up lactate for oxidative purposes, whereas MCT4 may be important for lactate efflux. Much work is still required to demonstrate the function of these MCT isoforms in muscle.

In keeping with the observation that lactate uptake is regulated in muscle, it is becoming clear that the expression of monocarboxylate transporters in muscle is subject to regulation. Endurance exercise and high-intensity chronic electrical stimulation enhance MCT1 expression in rat skeletal muscle [156,160], and exercise training also increases MCT1 levels in rat heart [160]. In humans, exercise training causes MCT1 up-regulation in muscle [161]. In contrast, muscle denervation reduces the expression of both MCT1 and MCT4 in muscle [156]. The mechanisms involved in the regulation of MCT1 and MCT4 in muscle remain to be determined.

FATTY ACID UPTAKE

Muscle utilizes large amounts of fatty acids: for example, a large percentage of the energy required to run a marathon race is derived from muscular oxidation of fatty acids, most of which come from the bloodstream. The mechanisms by which fatty acids cross the membrane are controversial. On the one hand, there is evidence from studies using unilamellar protein-free phospholipid vesicles that fatty acids cross membranes efficiently by simple diffusion [162]. On the other hand, some researchers have provided evidence for carrier-mediated uptake [163].

Fatty acid diffusion across membranes may be divided into several steps: (a) desorption of the fatty acid from albumin to which it is bound, (b) adsorption of an unbound monomeric form to the outer leaflet of the plasma membrane, (c) passage across the membrane (flip-flop), and (d) desorption from the cytosolic leaflet of the plasma membrane. Based on the utilization of unilamellar protein-free phospholipid vesicles, it has been reported that the rate constants for the adsorption of fatty acid monomers or for adsorption of fatty acids bound to albumin (at a high fatty acid/albumin ratio) to phospholipid bilayer vesicles are extremely high [164–166]. A more controversial aspect is the transmembrane movement, or flip-flop, of fatty acids across the membrane. Many studies use the pH-sensitive fluorophore pyranin to measure rates of flip-flop of fatty acids into phospholipid vesicles; some studies have shown that rates of flip-flop are high enough to account for fatty acid uptake into cells (rate constant for flip-flop $> 10\ s^{-1}$) [166,167]. In contrast, other studies in which flip-flop of long-chain anthroyloxy fatty acids was measured showed that the rate was low (rate constant $< 0.003\ s^{-1}$) [168]. The rates of fatty acid desorption from membranes are lower than the rates of adsorption, and depend on the fatty acid chain length and the degree of unsaturation [162]. However, there is controversy as to whether the rates of fatty acid desorption are lower or higher than the rate of flip-flop [162,168]. Thus these studies performed in model systems suggest that proteins are not essential for the movement of fatty acids across membranes, although there is discussion on rates and rate-limiting steps.

There is also growing evidence suggesting a carrier-mediated uptake of fatty acids into cells, based on: (a) the saturation kinetics of fatty acid uptake [169,170], which in rat cardiomyocytes accounts for 80% of palmitate uptake [169], (b) alterations to fatty acid uptake by covalent modification of proteins [171,172], and (c) the identification of several proteins that enhance fatty acid uptake [163]. In this connection, several membrane-associated proteins have been identified and are expressed in cardiac and skeletal muscles, namely a plasma membrane fatty acid-binding protein (FABPpm), a fatty acid translocase (FAT) and a fatty acid transport protein (FATP).

The plasmalemmal FABP, or FABPpm, is a peripheral membrane protein which is identical with the mitochondrial isoform of aspartate aminotransferase [173,174]. The cellular mechanisms by which the mitochondrial isoform of aspartate aminotransferase reaches the inner side of the plasma membrane and the domain(s) that permit binding to the plasma membrane are not understood. There are several indications that FABPpm participates in the uptake of fatty acids by cells. Thus antibodies directed against FABPpm diminished oleate uptake in isolated rat cardiomyocytes [175] and in 3T3-L1 adipocytes [176], and they also decreased palmitate uptake in preparations of giant sarcolemmal vesicles isolated from heart or skeletal muscle [177]. Inhibition with antibodies suggests that FABPpm may account for up to 50% of fatty acid uptake. Furthermore, 3T3 fibroblasts transfected with FABPpm/aspartate aminotransferase showed enhanced saturable oleate uptake in parallel with an increase in FABPpm bound to the plasma membrane [178]. The mechanisms by which FABPpm mediates fatty acid uptake remain unknown. In this regard, the determination of the precise role of FABPpm

in fatty acid transport across phospholipid vesicles is mandatory.

Recent reports indicate that expression of FABPpm is regulated in muscles. Thus the expression of FABPpm protein and mRNA is very high in heart, intermediate in red skeletal muscle and lower in white muscle, which parallels the capacity for fat oxidation [177,179,180]. Furthermore, endurance training, which enhances the capacity for fatty acid oxidation in muscle, also increases FABPpm levels [181].

FAT is an integral membrane protein which is considered to be the rat homologue of human CD36 [182] and which binds fatty acids. FAT has two predicted transmembrane domains and is oriented so that it has two short intracellular segments (Figure 2c). The remaining part of FAT is extracellular, containing 10 potential N-linked glycosylation sites, which may explain why the molecular mass of the isolated protein (88 kDa) is much greater than the mass deduced from the cDNA sequence (53 kDa). Studies on the purified bovine isoform of CD36 indicate that the six centrally clustered cysteines are linked by disulphide bonds, resulting in 1–3, 2–6 and 4–5 arrangements of the disulphide bridges [183]. Based on identity with the fatty acid binding site of heart-type FABP, a potential binding site for fatty acids may lie in its extracellular segment between residues 127 and 279 [184].

Several observations support a role for FAT in fatty acid uptake by cells, namely that (a) the expression of FAT in Ob17PY fibroblasts (which do not express endogenous FAT) enhances fatty acid uptake [185], and (b) transgenic mice showing muscle-specific overexpression of FAT are characterized by an enhanced muscle capacity to oxidize fatty acids in response to muscle contraction, and have low plasma concentrations of triacylglycerols and fatty acids [186]. As for FABPpm, the mechanism by which FAT contributes to fatty acid uptake and the extent of the participation of FAT in fatty acid uptake by tissues are unknown. However, FAT has a predicted structure which does not fit with that of a membrane transporter, so its role remains enigmatic. Perhaps germane to this issue, it has recently been reported that the expression of FAT in cardiac myocytes in culture does not enhance fatty acid uptake, suggesting that it requires additional protein components for its operation as a 'fatty acid translocase' [187]. In any case, as mentioned for FABPpm, studies in liposomes are required in order to analyse the precise role of FAT.

The FAT/CD36 gene was recently identified as a causal gene for insulin resistance in spontaneously hypertensive rats, based on quantitative trait loci mapping in the FAT gene, defective FAT gene expression in adipose tissue and a deletional mutation in FAT [188]. However, a further study demonstrated that a FAT gene defect was unlikely to be a major cause of the insulin-resistance phenotype in these rats, since no mutations in FAT were detected in the original spontaneously hypertensive rats [189].

FAT is expressed in muscles and is subject to regulation. During development, mRNA and protein levels are up-regulated in rat heart, which parallels an enhancement of the capacity of the heart to utilize fatty acids [190,191]. Chronic stimulation increases muscle fatty acid uptake by giant muscle vesicles, and enhances muscle FAT expression [192]. Furthermore, the expression of FAT protein and mRNA is very high in heart, intermediate in red skeletal muscle and lower in white muscle, which parallels their capacity for fat oxidation [177,179,180].

Mouse FATP was initially identified in an expression cloning strategy due to its ability to enhance fatty acid uptake [193]. Mouse FATP (FATP-1) encodes an integral membrane protein with six predicted membrane-spanning regions and three potential N-linked glycosylation sites; both the N- and C-terminal ends are oriented to the cytosolic side (Figure 2d). The human, rat and *Saccharomyces cerevisiae* orthologues of FATP have been isolated [194–196]. In fact, a total of five members of the family have been identified in murine tissues (FATP-1–FATP-5), and at least FATP-1, FATP-2 and FATP-5 enhance fatty acid uptake when expressed in COS cells [197]. In addition, FATP shows identity with rat very-long-chain fatty acyl-CoA synthases [196].

Stable overexpression of FATP confers an enhanced ability to take up fatty acids [193] and facilitates the uptake of saturated and monoenoic long-chain fatty acids with 14–22 carbon atoms, suggesting that it has broad specificity with respect to fatty acid chain length and degree of saturation [193]. The mechanism by which FATP facilitates fatty acid uptake is unknown. In this connection, it has been reported that murine FATP binds ATP [194], and studies with the yeast orthologue of FATP indicate that it displays very-long-chain fatty acid synthase activity, which might be essential for normal homoeostasis of very-long-chain fatty acids [196]. Comparisons of the sequences of murine, human and yeast orthologues have identified a conserved motif, IYTSGTTGXPK, which is also found in a number of proteins that form adenylated intermediates [194]. Substitution of alanine for serine-250 in this motif from murine FATP inhibits the uptake of long-chain fatty acids and ATP binding [194]. Taking all of these data together, it is reasonable to propose that FATP facilitates fatty acid uptake via membrane-associated fatty acyl-CoA synthase activity rather than by transport catalysis. However, as for FABPpm and FAT/CD36, it is crucial to analyse the role of FATP in vesicle models, as well as the effects of ablation of FATP on fatty acid homoeostasis *in vivo*.

FATP is expressed in cardiac muscle and skeletal muscle [182], and there are data indicating that its expression is regulated in adipocytes [198,199] and muscle [200]. Thus FATP mRNA levels are down-regulated in heart, skeletal muscle, adipose tissue, brain and other tissues in response to lipopolysaccharide (endotoxin) treatment *in vivo* under conditions in which fatty acid uptake is reduced [200].

CARNITINE UPTAKE

L-Carnitine (β-hydroxy-γ-trimethylaminobutyric acid) is an essential component in the transport of long-chain fatty acids into mitochondria, where they undergo β-oxidation. The enzyme carnitine palmitoyltransferase catalyses the conversion of carnitine and long-chain fatty acids into long-chain acylcarnitine esters, which are then transported to the mitochondrial matrix. L-Carnitine is synthesized endogenously from the essential amino acid lysine, with terminal methyl groups donated by *S*-adenosylmethionine. Methylation of the lysine side chain occurs when lysine is present in proteins. In humans the final reaction in the synthesis of carnitine is catalysed by a cytosolic hydroxylase, which operates mainly in liver, brain and kidney, and is not present in cardiac muscle or skeletal muscle [201,202]. In addition to endogenous synthesis, a notable amount of carnitine is provided by the diet, especially from meat and dairy products. Based on this, the requirements for carnitine in cardiac muscle and skeletal muscle are met by uptake from the extracellular milieu, and carnitine is normally maintained at a steady level in blood of approx. 50 μmol/l. It is eliminated as free carnitine or acylcarnitine almost exclusively by the kidneys, where it is filtered and reabsorbed in the proximal tubule. The concentrations of carnitine in human heart and muscle are 20–50-fold greater than in serum [201,202]. Failure of muscles to obtain sufficient carnitine results in cardiomyopathy or muscular weakness.

Carnitine uptake has been studied in different tissues and cells in culture, and kinetic data suggest the operation of several transporters. Liver and brain show a low-affinity (K_m in the range 2–10 mM), high-capacity carnitine uptake [203–205], while fibroblasts and heart cells have a high-affinity (K_m 5–10 μM), low-capacity system [206–209]. In addition, kidney brush-border membrane vesicles show both high-affinity and low-affinity components for carnitine uptake (K_m values of 17 μM and 15 mM respectively) [210]. Characterization of carnitine transport by renal brush-border vesicles shows Na^+-dependency, electrogenicity and inhibition by structural analogues, such as butyrobetaine, L-acetylcarnitine, trimethyl-lysine and D-carnitine [210].

Carnitine uptake by muscle cells varies with the differentiation state; thus human myoblasts in culture are characterized by high-affinity uptake (K_m 5 μM) [206,211]; muscle cell differentiation causes the appearance of a low-affinity component for carnitine uptake (K_m approx. 0.2 mM) [212,213]. Carnitine uptake by rat skeletal muscle is characterized by high K_m values, suggesting the operation of a low-affinity carnitine carrier [214,215]. Whether the low- and high-affinity carnitine transport components identified in different tissues or cells are the result of the expression of different transporters, or correspond to differential activity states of a single transporter, is unknown.

A human gene, named *OCTN2*, and a rat gene, *CT1*, showing high similarity (85 %) and which encode carnitine transporters have recently been isolated [216,217]. Human *OCTN2* has been mapped to chromosome 5q31.1–32 [216]. OCTN2 and CT1 belong to the family of organic cation transporters that includes OCT1, OCT2, OCT3 and OCTN1; OCTN2 is an integral membrane protein with 12 transmembrane domains and three potential glycosylation sites in the first extracellular loops, and both the N- and C-terminal ends are oriented intracellularly [218] (Figure 2e). OCTN2 is highly expressed in kidney, heart, skeletal muscle and placenta. Surprisingly enough, CT1 is mainly expressed in kidney, liver, intestine, testis and placenta; it is very weakly expressed in skeletal muscle, and no expression is detected in heart [218]. Whether this is due to differences between human and rat tissues, or to the fact that CT1 and OCTN2 are not orthologues, remains unknown.

Expression studies of OCTN2 in human embryonic HEK293 cells indicate that it mediates the uptake of carnitine against a concentration gradient, with an apparent Michaelis–Menten constant of 5–15 μmol/l [218,219]. One Na^+ ion is co-transported with one carnitine molecule [219]. Many zwitterionic drugs, such as cephaloridine, and many cationic drugs, such as verapamil and quinidine, inhibit OCTN2 transport activity [219]. In fact, OCTN2 is a Na^+-dependent carnitine transporter as well as a carrier for organic cations which operates in a Na^+-independent manner [220]. This is consistent with the fact that the transporters are organic cations are Na^+-independent [221]. The role of Na^+ is to enhance the affinity of OCTN2 for carnitine [220].

Primary systemic carnitine deficiency is an autosomal recessive disorder characterized by cardiomyopathy, skeletal myopathy, hypoglycaemia and hyperammonaemia. Several studies indicate that this disease is a consequence of a defect in carnitine transport [208], and this has also been reported in the mouse model, juvenile visceral steatosis (jvs mice) [222], which results in renal wastage and very low intracellular levels of carnitine. Recently it has been found that systemic carnitine deficiency is caused by mutations in the *OCTN2* gene that lead to deficient cellular carnitine uptake [223–228].

Several observations suggest the presence of additional carnitine transporters other than OCTN2 in muscle tissues. These include the fact that OCTN2 is a high-affinity carnitine carrier, whereas muscle also expresses a low-affinity carrier, and that myopathic carnitine deficiency is characterized by normal concentrations of carnitine in blood and in liver but low concentrations in muscle, giving rise to muscle weakness and lipid storage myopathy [229]. This raises the question of whether the low-affinity carnitine carrier is a separate entity, and suggests that a defect in this low-affinity carrier may be the cause of the primary myopathic carnitine deficiency.

AMINO ACID TRANSPORTERS

Skeletal muscle constitutes a large proportion of body weight in most mammals, including humans. As a consequence of its large mass, muscle is the body's main reservoir of amino acids and protein. Under anabolic conditions, muscle takes up amino acids from the extracellular space, in a pattern conforming to its protein composition. In contrast, in catabolic states or when protein synthesis is depressed, the pattern of amino acid release from muscle does not depend on the muscle's protein composition. Thus alanine and glutamine account for 40–50 % of the total amino acids released by human forearm and by the isolated perfused rat hindquarter, whereas they comprise at most 15 % of muscle protein [230–233], as a consequence of the *de novo* synthesis of alanine and glutamine in muscle [233–235]. In contrast with alanine and glutamine, several amino acids, such as leucine, isoleucine, valine, aspartate and glutamate, are released from skeletal muscle in lower amounts than would be expected from their content in muscle protein, probably reflecting their active catabolism in muscle [230,233]. Furthermore, skeletal muscle is a main site of metabolism of branched-chain amino acids; muscle takes up branched-chain amino acids and converts them into the 2-oxo acid species, which are released into the circulation [123,124,233,236–239] (see section on monocarboxylate transporters).

Other amino acids, such as glycine, cysteine, serine, threonine, methionine, proline, lysine, arginine, histidine, phenylalanine, tyrosine and tryptophan, can be either removed from the extracellular space by muscle for incorporation into protein or released upon proteolysis [123].

This complex pattern of amino acid influx and efflux in muscle is catalysed by many different amino acid carriers. In the early 1960s, transport systems for amino acids with different substrate specificities were identified in mammalian cells, which revealed the existence of general properties of mammalian amino acid transport, such as stereospecificity and broad substrate specificity. Since these initial studies, the main functional criteria used to define amino acid transporters are the type of amino acid carried (zwitterionic, cationic or anionic) and the thermodynamic properties of the transport (active transport, facilitative diffusion). This functional classification is still used (Table 1), since structural information on mammalian amino acid transporters is not complete.

Amino acid transport agencies in muscle

Studies performed in isolated perfused rat hindquarter, inincubated muscle preparation and in muscle cells in culture have revealed a number of amino acid transport systems (Table 1). Most neutral amino acids are transported in muscle via four distinct transport agencies, i.e. systems A, ASC and N^m (which show Na^+-dependence) and system L (which is Na^+-independent) [240–244]. There is also evidence for the operation in muscle of transport of the β-amino acid, taurine, by a Na^+-dependent carrier [245]. Cationic amino acids are transported in muscle by the Na^+-independent system y^+ [246], and aspartate and glut-

Table 1 Amino acid transport systems present in the plasma membrane of mammalian cells

Abbreviations: MeAIB, methylaminoisobutyric acid; BCH, α-aminoendobicyclo-[2,2,1]-heptane-2-carboxylic acid.

Transporter	Properties	Isolated cDNAs	Transport activity in muscle
Zwitterionic amino acids			
Sodium-dependent			
A	Serves mainly for small amino acids. Highly regulated. Tolerates an N-methyl group. Sensitive to pH changes. Trans-inhibition associated. Widespread.		Yes
ASC	Excludes N-methylated amino acids. Trans-stimulation associated. Widespread.	ASCT1, ASCT2	Yes
N	For Gln, Asn and His. Sensitive to pH changes. Restricted to hepatocytes. Variant N^m in muscle.	SN1	Yes
BETA	Transports β-Ala, taurine and GABA. Chloride-dependent. Variants known. Widespread.	Series GAT-1 to GAT-3; BGT-1; TAUT	Yes
GLY	Transports Gly and sarcosine. Chloride-dependent. Variants known. Present in several tissues.	GLYT1, GLYT2	
IMINO	Handles proline, hydroxyprolines and N-methylated glycines. Interacts with MeAIB. In intestinal brush-border membranes.		
PHE	Phe and Met. In brush-border membranes.		
B^o	Broad specificity for most zwitterionic amino acids, including branched aromatic ones. Accepts BCH but not MeAIB. In brush-border membranes. Most probably identical to system NBB (renamed B).		
Sodium-independent			
L	Mainly for bulky-side-chain amino acids. Trans-stimulated. Bicyclic amino acids as model substrates. Variants described. Widespread.	4F2hc/LAT-1, LAT-2	Yes
Cationic amino acids			
Sodium-dependent			
$B^{o,+}$	Broad specificity for zwitterionic and dibasic amino acids. Accepts BCH but not MeAIB. In blastocysts, *Xenopus* oocytes and probably also brush-border membranes.	$B^{o,+}$-AT	
Sodium-independent			
b^+	Cation preferring. Does not interact with homoserine even in the presence of sodium. Variants known.		
y^+	Handles cationic and zwitterionic amino acids with sodium. Variants known. Sensitive to *N*-ethylmaleimide. Widespread.	CAT-1 to CAT-4	Yes
y^+-L	Handles cationic amino acids, and zwitterionic amino acids with high affinity only with sodium. Insensitive to *N*-ethylmaleimide. In erythrocytes and placenta.	4F2hc/y^+-LAT-1, y^+-LAT-2	
$b^{o,+}$	Like $B^{o,+}$ but limited by positions of branching. Not inhibited by BCH. In blastocysts and brush-border membranes.	rBAT/$b^{o,+}$-AT	
Anionic amino acids			
Sodium-dependent			
X^-_{AG}	Similarly reactive with L-Glu and D- and L-Asp. K^+-dependent. Widespread.	EAAT1 to EAAT5	Yes
Sodium-independent			
x^-_C	Cystine competes and exchanges with Glu. In heptocytes and fibroblasts. Electroneutral.	xCT	Yes

amate are taken up in muscle cells and in the perfused hindquarter via Na^+-dependent and Na^+-independent mechanisms (Table 1) [246,247]. Because of the limitations imposed in the studies using skeletal muscle, it is likely that there are more amino acid agencies in this tissue than the ones that have been identified by kinetic means.

There is substantial evidence that system A transport activity is regulated in skeletal muscle. Thus both insulin and exercise stimulate system A activity in muscle in an acute manner [240,242,248–253]. The stimulatory effect of insulin has also been described in human muscle [254]. In addition, amino acid starvation up-regulates system A activity in muscle by a mechanism that requires both protein synthesis and intact microtubular function [255]. The effect of insulin on system A in muscle is characterized by an enhanced V_{max} of transport, is independent of protein synthesis and of the Na^+-electrochemical gradient, and does not require intact microtubular function [253,255,256], suggesting a possible direct effect of insulin on the system A transporter. System A activity in skeletal muscle is also regulated acutely by phospholipase C and by vanadate [257,258], and the latter compound may activate system A by increasing the intracellular pH [258]. System A may also be regulated by long-term mechanisms; in this regard, it has been reported that streptozotocin-induced diabetes causes up-regulation of system A activity in both red and white muscle [259]. The precise nature of the mechanisms that regulate system A is unknown, since identification of the system A gene has not been reported.

The activity of system N^m is responsible for the transport of glutamine and asparagine in a sodium- and pH-dependent manner, with stoichiometry of 1 Na^+:1 glutamine [243,246]. N^m is responsible for both the influx and the efflux of glutamine in muscle, and is subject to regulation. Glutamine transport in muscle is up-regulated in response to incubation in a glutamine-free medium [260], i.e. it undergoes adaptive regulation. Insulin stimulates N^m activity in muscle by a mechanism that is characterized by an enhanced V_{max} and by sensitivity to inhibition by cycloheximide [261]. System N^m transport activity is also subject to rapid regulation after changes in cell volume. Thus hypo-osmotic swelling causes stimulation and hyperosmotic shrinkage induces inhibition of Na^+-dependent glutamine uptake in cultured muscle cells, and these effects are blocked by the phosphatidylinositol 3-kinase inhibitor, wortmannin [262,263]. The cloning of one N system, named SN1, has recently been reported [264]; this may accelerate the identification of the N^m carrier and thus the molecular analysis of the regulatory mechanisms that operate in muscle.

Another system that is subject to regulation in muscle is the transport of the β-amino acid taurine. Taurine uptake shows sodium-dependence in muscle and cardiomyocytes, and fibre type dictates differences in uptake, so that taurine uptake is greater in red than in white muscle [265]. In addition, chronic electrical stimulation of the extensor digitorum longus stimulates taurine transport [266]. In keeping with the role of taurine as an organic osmolyte, hypotonicity strongly activates taurine efflux from muscle cells in culture [267].

The transport of anionic amino acids is also regulated in muscle. Thus insulin increases glutamate uptake by human forearm muscle [268]. There are differences in the agencies that participate in glutamate transport in the muscle fibre or in cultured muscle cells and, whereas glutamate transport in the perfused rat hindlimb shows stereospecificity and sodium-independence [246], glutamate is transported in muscle cells in culture by Na^+-dependent (probably X^-_{AG}) and Na^+-independent (probably x^-_C) agencies [247]. The sodium-dependent component of glutamate transport is up-regulated by incubation in the

Table 2 Families of amino acid transporters

In the family of heteromultimeric amino acid transporters, light subunits LAT-1, LAT-2, y^+-LAT-1, y^+-LAT-2 and xCT are believed to associate with heavy subunit 4F2hc, whereas light subunit $b^{0,+}$-AT associates with heavy subunit rBAT. See the text for further details.

Transporter	Expression in muscle
(1) Transporters of cationic amino acids	
CAT-1	Yes
CAT-2	Yes
CAT-2a	Yes
CAT-3	
CAT-4	
(2) Sodium- and chloride-dependent amino acid transporters	
GAT-1	
GAT-2	
GAT-3	
BGT-1	Yes
TAUT	Yes
GLYT1	
GLYT2	
PROT	
$B^{0,+}$-AT	
(3) Sodium-dependent transporters for anionic and zwitterionic amino acids	
EAAT1 (rat GLAST)	Yes
EAAT2 (rat GLT-1)	
EAAT3 (rabbit EAAC1)	Yes
EAAT4	
EAAT5	Yes
ASCT1	Yes
ASCT2	Yes
(4) Heteromultimeric amino acid transporters	
Heavy subunits	
4F2hc	Yes
rBAT	
Light subunits	
LAT-1	
LAT-2	Yes
y^+-LAT-1	
y^+-LAT-2	
xCT	
$b^{0,+}$-AT	
(5) Sodium- and proton-dependent vesicular neurotransmitter transporters	
SN1	

presence of glutamine by a mechanism that is sensitive to inhibitors of both transcription and protein synthesis [247].

Expression of amino acid transporters in muscle

The primary sequences of most amino acid transport agencies in mammalian cells have been identified. At present, amino acid transporters are categorized in five different gene families, which are presented in Table 2: (1) family of sodium-independent transporters of cationic amino acids; (2) superfamily of sodium- and chloride-dependent neurotransmitter transporters, which also includes amino acid transporters; (3) superfamily of sodium-dependent transporters for anionic and zwitterionic amino acids; (4) family of heteromultimeric amino acid transporters, constituted by two distinct subunits (Table 2); and (5) the recently identified SN1 transporter, which belongs to the family of sodium- and proton-dependent vesicular neurotransmitter transporters, and which is not expressed in muscle [264]. For a thorough discussion of amino acid transporter families identified up to 1998, see [15].

Most of the work performed up to now in this field has entailed the molecular identification of the transporters, functional studies after overexpression in cells, and a few studies on structure/function relationships and topological properties. Almost nothing is known regarding the specific function of these transporters in muscle. In fact, the reported information is essentially limited to the expression of mRNAs for transporters in muscle, so that in many instances we do not even know whether the transporter is expressed in the muscle fibre or in cardiac myocytes.

Family of sodium-independent transporters of cationic amino acids

These carriers catalyse the sodium-independent transport of cationic amino acids (L-arginine, L-lysine or L-ornithine), with high affinity (K_m in the micromolar range), as is the case for CAT-1 or CAT-2, or with low affinity (K_m in the millimolar range) by CAT-2a [269–271]. CAT transporters comprise the functionally defined cationic amino acid system y^+ [269–272]. Based on Northern blot assays, cardiac muscle and skeletal muscle are known to express CAT-1, CAT-2 and CAT-2a [269].

CAT-2 and CAT-2a are isoforms derived from mutually exclusive alternative splicing of the primary transcript, and differ only in a 41–42-amino-acid segment located in the intracellular loop between transmembrane domains VIII and IX of the 14-transmembrane-domain model (Figure 2f) [271].

The transport of cationic amino acids via CAT-1 is voltage-dependent. Hyperpolarization increases the V_{max} and decreases the K_m for influx, with the reverse effects for efflux [273]. For CAT-1, CAT-2 and CAT-2a, transport is electrogenic (positive charge follows the cationic amino acid flux) and stereospecific [273–275]. In addition, CAT-1, CAT-2 and CAT-2a present variable trans-stimulation of arginine uptake, although trans-stimulation of CAT-2a is minimal [276–278].

Structural information on CAT transporters is scarce, and most available data come from the analysis of mouse CAT-1. CAT transporters lack a characteristic signal peptide, and in consequence the N-terminus is considered to be cytosolic [270,272]. Hydrophobicity profiles predict either 12 or 14 transmembrane-spanning domains, although most data support the 14-transmembrane-domain model [15,279,280] (Figure 2f).

The specific role of the different CAT transporters expressed in muscle, i.e. CAT-1, CAT-2 and CAT-2a, is unknown. However, based on the kinetics of the different CAT transporters and on the plasma and intracellular concentrations of cationic amino acids, it is reasonable to assume that CAT-1 and CAT-2, if indeed they are expressed in muscle cells, participate in the uptake of cationic amino acids, whereas the low-affinity transporter CAT-2a might be more active in the efflux of lysine and arginine. In this regard, it is known that there is differential regulation of these carriers. Thus fasting or stress induced in mice by surgical trauma, situations characterized by muscle protein catabolism and release of cationic amino acids from muscle, enhance CAT-2a gene expression in skeletal muscle in the absence of alterations in CAT-1 or CAT-2 expression [281,282]. Whether the up-regulation of CAT-2 gene expression contributes to muscle protein catabolism by enhancing the release of cationic amino acids from muscle into the extracellular milieu or, alternatively, whether CAT-2a contributes to the buffering of protein catabolism by enhancing the expression of a low-affinity transporter, remains unknown.

Family of sodium- and chloride-dependent amino acid transporters

Different transporters belonging to this family have been isolated, and catalyse the uptake of amino acids such as GABA, betaine, taurine, glycine and proline (Tables 1 and 2) (for a review, see [15]). The superfamily also includes transporters for neurotransmitters such as dopamine, 5-hydroxytryptamine (serotonin) and noradrenaline, as well as the creatine transporter reviewed above [283,284]. Sodium- and chloride-dependence has been reported for all these transporters. Skeletal muscle expresses substantial levels of mRNAs corresponding to BGT-1 and TAUT [285,286]; however, no further information is available. BGT-1 catalyses the transport of GABA, betaine and L-proline, as assessed in *Xenopus* oocytes, COS cells and 9HTEo cells [285,287,288]. The TAUT transporter takes up taurine and β-alanine with high affinity after expression in *Xenopus* oocytes, COS7 cells and HeLa cells [289–292]. The function of these two transporters in muscle remains unknown; however, based on the fact that taurine and betaine are non-perturbing osmolytes, they may counteract differences in osmolarity between muscle cells and the extracellular medium. In this regard, the activity of TAUT and BGT-1 transporters is regulated by tonicity in MDCK (Madin–Darby canine kidney) renal cells, RAW 264.7 cells and H4IIE rat hepatoma cells [293–295].

The amino acid transporters in this family show high identity (at least 40 %) in their primary structure, with nearly 150 well-conserved amino acid residues [15]. As general features, all these transporters lack a signal peptide, and show a good prognosis for 12 transmembrane domains, with the N- and C-termini located intracellularly, and for between one and four putative N-glycosylation sites between transmembrane domains 3 and 4, as shown for the creatine transporter CRT1 (Figure 2a). This prognosis has been validated in different studies for GAT-1 and GLYT1 [296,297].

Family of sodium-dependent transporters for anionic and zwitterionic amino acids

This family comprises five transporters of anionic amino acids (EAAT1–EAAT5) and two transporters of zwitterionic amino acids (ASCT1 and ASCT2) (Table 2), all of which are sodium-dependent (for a review, see [15]; also see [15] for a detailed explanation of the nomenclature used by researchers). On the basis of sequence identity, this family can be subdivided into two subfamilies: the human EAAT isoforms (the anionic amino acid transporters), which show 36–65 % amino acid sequence identity between them, and the human zwitterionic amino acid transporters ASCT1 and ASCT2, which show 57 % identity between them. The characteristics of their expressed transport activity suggest that the EAAT1–EAAT5 isoforms are variants of system X^-_{AG}, and that ASCT1 and ASCT2 are variants of system ASC.

Northern blot analysis indicates that skeletal muscle expresses EAAT1 and EAAT3 [298,299]; EAAT5 may also be expressed in muscle, although the bands shown in Northern blot assays for skeletal muscle and heart were near 2.0 kb, whereas the EAAT5 cloned from retina was 3.1 kb in size [300]. The precise distribution of EAATs in muscle tissue is unknown, although they may participate in the muscle uptake of anionic amino acids. Muscle also expresses ASCT1 and ASCT2, which may contribute to the ASC transport activity reported in this tissue [301,302].

The transporters of this family show potassium-dependence in addition to sodium-dependence when expressed in different cell systems, with the possible exception of the transporters of zwitterionic amino acids [300,303,304]. Thus the glutamate transporters are sodium co-transporters and potassium countertransporters. ASCT1 and ASCT2 are probably electroneutral sodium-dependent amino acid exchangers that do not interact with potassium. Surprisingly enough, some of these transporters (EAATs and ASCTs) function as a substrate (amino acid and sodium)-gated chloride channel in addition to their amino acid transport mode of action [300,303,305]. However, the molecular basis for this ligand-gated chloride channel activity remains unknown.

The main common features of the members of this family are: (1) the absence of a cleavable signal sequence, suggesting a cytosolic localization of the N-terminus, (2) the presence of two canonical sites for N-linked glycosylation on the extracellular loop between transmembrane domains 3 and 4, and (3) the presence of six highly conserved putative membrane-spanning domains in the N-terminal half of the protein (Figure 2g). There has been controversy with regard to the topology of these transporters, which has been resolved for rat EAAT2 (named GLT-1) by using single cysteine mutants and assaying biotinylation [306]. Data indicate that rat EAAT2 has eight transmembrane domains; between the seventh and the eighth membrane-spanning domains, a structure reminiscent of a pore loop and an outward-facing hydrophobic linker are positioned [306]. This topology is presented for the isoform EAAT1, which is expressed in skeletal muscle, in Figure 2(g).

Family of heteromultimeric amino acid transporters

Very recently, a new family of amino acid transporters has been identified. All members of this family are constituted by light subunits, with a structure compatible with that of a transporter, which must complex with a heavy subunit, either 4F2hc or rBAT, in order to be fully active [307–316]. Several members of this family have been identified, and they show many different types of sodium-independent amino acid exchange, which permits the uptake of zwitterionic, anionic or cationic amino acids (Table 1). Thus LAT-1 and LAT-2 are variants of L-type transport activity for neutral amino acids [307,308,311–313], y^+-LAT-1 and y^+-LAT-2 are variants of the transport system y^+-L for cationic amino acids [309,310], $b^{0,+}$-AT functions like the transport system $b^{0,+}$ with affinity for both zwitterionic and cationic amino acids [314,315], and xCT represents the transport activity system x^-_c for anionic amino acids [316]. It is likely that additional isoforms will be found in mammalian tissues. Molecular genetics has revealed that rBAT, $b^{0,+}$-AT and y^+-LAT-1 are crucial in renal function, and mutations in these genes have been identifed in cystinuria and in lysinuric protein intolerance [314,315, 317–319].

The only isoform of this family described so far in skeletal muscle is LAT-2 [311]. Based on the fact that co-expression of LAT-2 and 4F2hc in *Xenopus* oocytes induces L-type amino acid transport activity characterized by broad specificity for small (glycine, alanine, serine, threonine and cysteine) and large (leucine, isoleucine, phenylalanine, methionine, tyrosine, histidine, tryptophan, valine, asparagine and glutamine) zwitterionic amino acids [311], LAT-2 may contribute to the sodium-independent uptake or release of these amino acids in muscle. Because of the quantitative importance of alanine and glutamine release by muscle under a variety of physiological conditions, we postulate that LAT-2 may contribute to this function in muscle. In addition, since LAT-2 works as an exchanger, it is likely that alanine or glutamine release is concomitant with the uptake of branched-chain amino acids.

Human LAT-2 shows amino acid sequence identity of 50, 44 and 45 % with human LAT-1, y^+-LAT-1 and y^+-LAT-2 respectively [311]. Hydrophobicity studies suggest the presence of

12 transmembrane domains, with both the N- and C-terminal segments located intracellularly (Figure 2h). There is only one putative N-glycosylation site, between putative transmembrane segments VIII and IX; however, according to predictions, this segment is intracellular and additionally is not glycosylated in an *in vitro* microsomal system [308], which is in keeping with the observations for the rest of the family. No other experimental data have been produced to support the topological predictions.

LAT-2 interacts with 4F2hc (4F2 cell surface antigen heavy chain), a ubiquitous membrane protein, and the formation of this complex (with unknown stoichiometry) permits the arrival of LAT-2 at the cell surface; expression of a tagged version of LAT-2 in *Xenopus* oocytes in the absence of 4F2hc resulted in its intracellular accumulation and non-arrival at the cell surface [311]. The association of y^+-LAT-1 with 4F2hc is linked by disulphide bridges with residue cysteine 109 of human 4F2hc [311]. Protein 4F2hc has no membrane leader sequence, so its N-terminus is located intracellularly and a single transmembrane domain is predicted, and biochemical evidence suggests that 4F2hc is a type-II integral membrane N-glycoprotein [320–322].

PROSPECTS

A major task in the field of plasma membrane transporters during the last few years has been the identification of the genes encoding them. In fact, there is still much work to be done on the molecular biology of membrane transporters, since genes encoding several important ones, such as amino acid transporters A and N^m, have not yet been identified, and more carnitine transporters or isoforms for known transporters may be identified in the future. Glucose transporters were identified in the mid to late 1980s, and the study of their biological properties has received much more attention because of their relationship with insulin action and insulin resistance. However, knowledge on the biology of membrane transporters other than glucose transporters is extremely scarce. In consequence, for all the identified membrane transporters with a large impact in metabolism, it is time to elucidate the precise role that these molecules play in cells, and therefore their role in muscle metabolism. Important questions that must be addressed relate to the understanding of the cell biology of these proteins, as well as their specific function. This will be accomplished by analysing the cell biology of the proteins, and by the generation of transgenic animals where the transporter is overexpressed in a tissue-dependent manner, and by the ablation *in vivo* of specific genes in a tissue-specific or time-dependent manner. This is not an easy task, especially in skeletal muscle, due to the inherent difficulty of working with this tissue and the lack of good cell models; for example, clearly much more is known about glucose transporters in adipose cells than in skeletal muscle, mostly due to the reasons specified above.

These studies aimed at the assessment of cellular function may also identify new target molecules for therapy. There is a need for new drugs to ameliorate the insulin resistance of Type-II diabetes mellitus, in the treatment of obesity, to prevent and reverse cachexia induced by many different conditions, and to reverse muscle atrophy induced by immobilization or due to aging. We now know that the GLUT4 glucose transporter is an appropriate target for therapy in insulin-resistant states, and much effort is being made to identify molecules that regulate the presence of this transporter at the cell surface. Perhaps some transporters reviewed in this article may regulate the size of the muscle fibre, or may permit the entry of molecules that are important for the proliferation or the differentiation of muscle cells.

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