

Glucose transporters in obesity

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Les transporteurs de glucose dans l'obésité

RÉSUMÉ

L'obésité, pathologie fréquente dans les sociétés industrialisées, est caractérisée par un sur-développement du tissu adipeux. Nous avons étudié un modèle animal d'obésité, le rat Zucker génétiquement obèse. Dès l'âge de 30 jours, les cellules adipeuses des rats mutants transportent beaucoup plus de glucose que celles des témoins normaux des mêmes portées. Cette anomalie repose sur une augmentation du nombre des transporteurs de glucose et plus particulièrement sur une surexpression de l'isoforme GLUT4. La régulation de l'expression de GLUT4 par le génotype (*fa*) est spécifique du tissu adipeux. De plus, nous avons montré récemment qu'elle passe par une suractivation du promoteur. La surexpression de GLUT4 permet une entrée massive de glucose dans les cellules adipeuses, favorisant une lipogénèse excessive. Ultérieurement, les niveaux adipocytaires de GLUT4 diminuent, en conjonction avec l'apparition d'une insulino-résistance. Chez l'homme obèse, la diminution de l'expression de GLUT4 dans le tissu adipeux est aggravée par le diabète non-insulino dépendant (DNID), un défaut qui concerne également une autre isoforme GLUT5, transporteur de fructose. L'implication de GLUT5 dans l'utilisation des hexoses par la cellule adipeuse et son rôle dans le DNID restent à déterminer.

Obesity is a common human health problem in industrialized societies and is characterized by increased adipose tissue mass. Obesity is highly correlated with insulin resistance and non-insulin-dependent diabetes mellitus (NIDDM). However, the molecular mechanisms involved in these syndromes are still poorly understood. In addition to defects in systems which regulate food intake and energy expenditure, one cellular site at which potential abnormalities might reside is the adipose cell.

The adipose cell is devoted to storing energy in the form of triacylglycerols, which are synthesized through two major pathways: lipogenesis from glucose and esterification of free fatty acids. The latter pathway also utilizes glucose for the production of the C₃ skeleton of glycerol. The relative contributions of these pathways to lipid formation varies with pathophysiological conditions, including ageing. A major control point for both is the transport of glucose into the adipose cell. Glucose uptake is mediated by specialized integral membrane proteins, the facilitative glucose transporters (GLUT). Until now, six isoforms have been discovered, each having specific tissue distribution and kinetic properties. At least three GLUT isoforms are expressed in adipose cells: GLUT1, which is also present in many other tissues; GLUT4, which is expressed almost

exclusively in tissues where glucose transport activity is acutely regulated by insulin (i.e. adipose tissues and muscle); GLUT5, which has been detected in human adipose cells and is thought to transport fructose preferentially.

The present review focuses on the alterations in glucose transport and glucose transporter expression in adipose cells in an animal model of obesity, the Zucker fatty rat. Recent data obtained from obese human subjects with or without NIDDM will also be presented.

AN ANIMAL MODEL OF OBESITY: THE ZUCKER FATTY RAT

The genetically-obese *falfa* rat, first described by Zucker & Zucker (1961), expresses an autosomal recessive mutation, the *fa* gene. The mutation has been mapped recently to chromosome 5 (Truett *et al.* 1991), but the protein remains totally obscure. The rats develop a syndrome with multiple metabolic and hormonal disorders which closely resembles human obesity. Hyperphagia, hyperinsulinaemia and hypertriacylglycerolaemia are features common to both species. Therefore, the unravelling of biochemical defects in these rats might lead to a better understanding of human obesity.

The obesity of the Zucker rat is of early onset. In suckling obese pups (16 d of age), adipose tissue mass and fat cell size are already twice those observed in lean littermates; however, insulinaemia is similar in both genotypes (Table 1). At weaning (about 25 d of age), the rate of adipose tissue growth accelerates dramatically, along with the emergence of hyperphagia and hyperinsulinaemia. By 30 d of age, the obese rats display 4–5-fold higher adipose tissue mass and fat cell size than lean littermates and are markedly hyperinsulinaemic (Table 1).

GLUCOSE TRANSPORT ACTIVITY IN ADIPOSE CELLS

Cushman *et al.* (1978) and Czech *et al.* (1978) have previously shown an increase in insulin's stimulatory effect on glucose transport in isolated epididymal adipose cells from

Table 1. *General characteristics of young Zucker rats*

(Mean values with their standard errors for eighteen determinations)

Age (d) . . .	16				30			
	Lean (<i>Falfa</i>)		Obese (<i>falfa</i>)		Lean (<i>Falfa</i>)		Obese (<i>falfa</i>)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body wt (g)	24.5	0.30	26.2*	0.27	58.2	1.3	63.7*	1.3
Inguinal adipose pads weight (g)	0.13	0.01	0.33*	0.01	0.52	0.02	1.81*	0.12
Adipose cell size (μg lipid/cell)	0.016	0.002	0.045*	0.004	0.040	0.003	0.18*	0.01
Adipose cell surface area ($\mu\text{m}^2 \times 10^{-3}$)	3.0	0.26	6.1*	0.39	4.7	0.31	14.5*	0.30
Plasma glucose (mM)	7.94	0.12	8.44*	0.23	9.16	0.38	8.65	0.15
Plasma insulin (nM)	0.15	0.02	0.17*	0.01	0.28	0.03	0.74*	0.13

Mean values for obese rats were significantly different from those of lean rats: * $P < 0.01$.

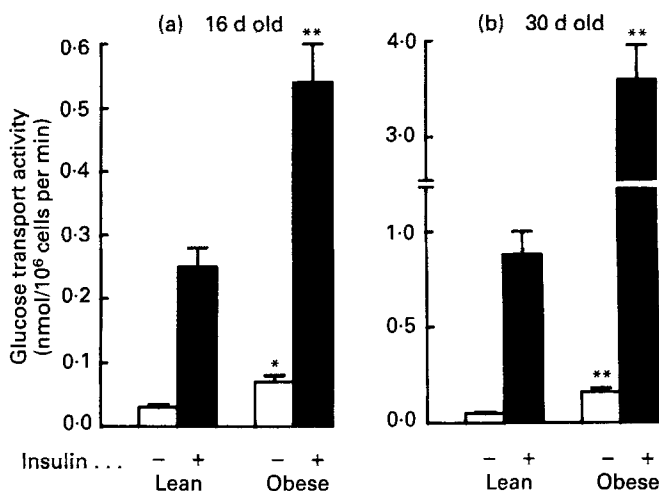


Fig. 1. Glucose transport activity in isolated adipose cells from young lean and obese Zucker rats at 16 and 30 d of age. (□), Basal (without insulin); (■), + insulin (0.72 nM). Values are means with their standard errors represented by vertical bars.

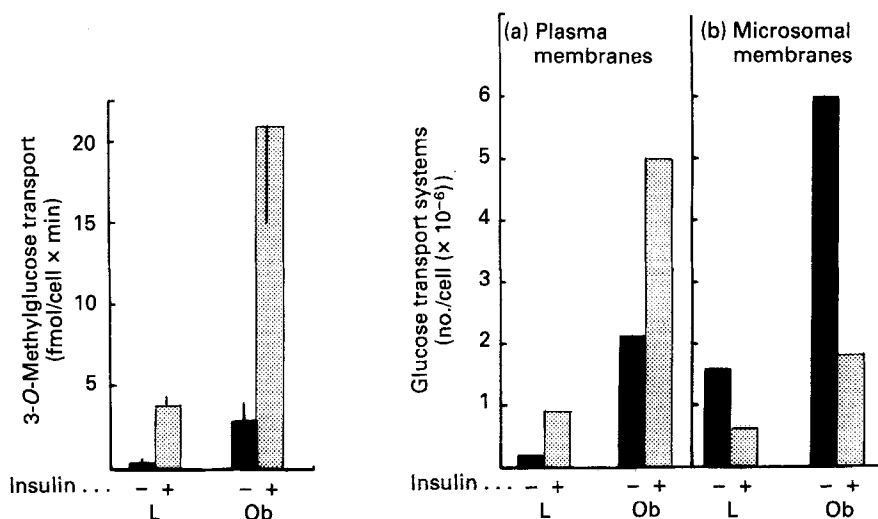


Fig. 2. Glucose transport activity and the number of glucose transporters measured by cytochalasin-B binding in adipose cells from lean (L) and obese (Ob) 30-d-old Zucker rats. (■), Basal (without insulin); (▨), + insulin (0.72 nM). Values are means with their standard errors represented by vertical bars.

6- and 10-week-old obese Zucker rats. In good agreement, we have observed that inguinal adipose cells from 30-d-old obese Zucker rats exhibit a several-fold increase in glucose transport activity as compared with lean littermates (Fig. 1). These observations are consistent with our *in vivo* studies demonstrating that the hypertrophied inguinal adipose tissue from obese rats utilizes 10-fold more glucose than the adipose tissue from lean littermates (Krief *et al.* 1988). In addition, enhanced glucose transport activity of

adipose cells, although less marked, is already apparent in suckling obese pups at 16 d of age (Fig. 1).

In a first attempt to delineate the molecular mechanisms underlying the enhanced glucose transport in the obese rat fat cells, we measured the number of glucose transporters (Guerre-Millo *et al.* 1985). Using the cytochalasin-B binding method, we were able to determine that the number of glucose transporters in the low-density microsomes is dramatically increased in the obese rats (Fig. 2). In adipose cells, this intracellular membrane fraction represents a storage compartment from which glucose transporters are translocated to the plasma membrane in response to insulin binding to its membrane receptor. The increased size of the intracellular reserve results in more glucose transporters reaching the plasma membrane following insulin stimulation. It is apparent from Fig. 2 that a good correlation is observed between glucose transport activity in lean and obese rat fat cells and the number of glucose transporters at the cell surface. This was the first demonstration of an increased intracellular pool of glucose transporter in an insulin-hyper-responsive cell. Interestingly, this situation is the opposite of that occurring in insulin-resistant adipose cells from diabetic or fasted rats (for review, see Kahn, 1992).

OVEREXPRESSION OF GLUT4 IN ADIPOSE CELLS

The cytochalasin-B binding method does not discriminate between glucose transporter isoforms. When it was discovered that two transporters, GLUT1 and GLUT4, were expressed in rat adipose cells, the question arose as to which isoform is overexpressed in the young obese Zucker rat. Using isoform-specific antibodies, we determined (Hainault *et al.* 1991) that the concentration of GLUT4 was significantly higher in membranes prepared from obese-rat adipose cells than in those from lean rats, whereas the concentration of GLUT1 was similar at 16 d of age or lower at 30 d of age. With larger cells in the obese rat, total cellular content of GLUT4 was 2.4- and 4.5-fold higher at 16 and 30 d of age respectively. In contrast, the amounts of GLUT1 per cell were similar in the two genotypes. As shown in Fig. 3, the increase in insulin-stimulated glucose transport activity in obese, relative to lean, rat fat cells is fully accounted for by the increase in the cellular content of GLUT4. In addition, the overexpression of GLUT4 is specific to white adipose tissue, as GLUT4 levels are unchanged in skeletal muscle (Hainault *et al.* 1991) and tend to be decreased in brown adipose tissue in obese *v.* lean pups (Charron *et al.* 1995).

Hyperinsulinaemia is already apparent in 30-d-old obese Zucker rats (Table 1), suggesting that this might be the underlying cause of the differential expression of GLUT isoforms in adipose cells as compared with normo-insulinaemic lean controls. Indeed, a similar pattern of regulation, in which an increase in GLUT4 expression is associated with unchanged GLUT1 is also observed in adipose cells from rats whose insulinaemia was increased by refeeding after a fast or insulin treatment of streptozotocin diabetes (Kahn, 1992). However, we observed that the overexpression of GLUT4 is already present at 16 d of age in normo-insulinaemic obese pups (Fig. 3). This suggests that the *fa*-dependent up-regulation of GLUT4 expression is independent of hyperinsulinaemia.

These observations strongly suggest that early overexpression of GLUT4 in adipose tissue resulting in increased glucose uptake is involved in the development of obesity. This alteration could be responsible for the preferential shunting of glucose into adipose

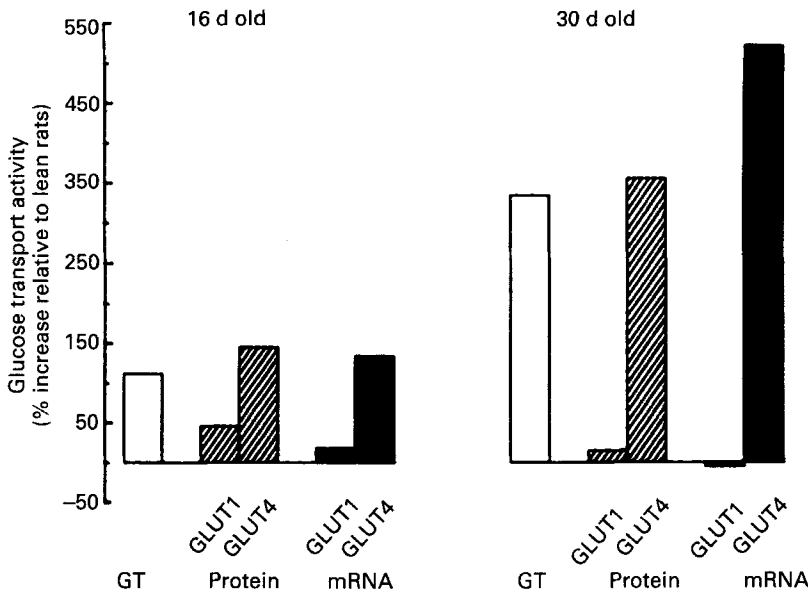


Fig. 3. Genotype effect on insulin-stimulated glucose transport (GT, □), GLUT1 and GLUT4 protein per adipose cell (▨) and GLUT1 and GLUT4 mRNA per fat pad (■). Results in obese rats at 16 and 30 d old are expressed as the percentage increase relative to corresponding values for lean rats.

tissue triacylglycerols which characterizes the obese phenotype in the Zucker rat. Interestingly, increased GLUT4 expression in adipose cells has been reported in other rodent models of obesity such as ventromedial hypothalamus-lesioned rats (Cousin *et al.* 1992) and mice made obese by gold thioglucose injection (Le Marchand-Brustel *et al.* 1990). More recently, the availability of several lines of transgenic mice overexpressing GLUT4 in adipose tissue, which develop obesity, has reinforced the potential role of GLUT4 overexpression in fat (Liu *et al.* 1993; Shepherd *et al.* 1993).

DECREASED EXPRESSION OF GLUT4 IN AGED OBESE RATS

Although a marked overexpression of GLUT4 in adipose cells is observed early in life, i.e. in the dynamic phase of the development of obesity, it has been shown that 20-week-old obese Zucker rats have less GLUT4 per cell than lean controls (Pedersen *et al.* 1992). This suggests that eventually factors which down-regulate GLUT4 overcome the genotype effect. Relevant to these observations, the adipose cell GLUT4 is decreased in the aged (12-month-old) obese Sprague-Dawley rat, along with a decreased intracellular pool of transporters and a decreased insulin effect on glucose transport, as compared with young lean controls (Hissin *et al.* 1982; Ezaki *et al.* 1990). The factors responsible for the down-regulation of GLUT4 in adult or aged obese rats, which could be related to long-term hyperinsulinaemia or hyperlipaemia, remain unknown.

MOLECULAR MECHANISMS OF ENHANCED GLUT4 EXPRESSION

As shown in Fig. 3, the levels of GLUT1 and GLUT4 mRNA closely parallel the levels of the proteins, at both ages and in both genotypes. Similarly, Pénicaud *et al.* (1991)

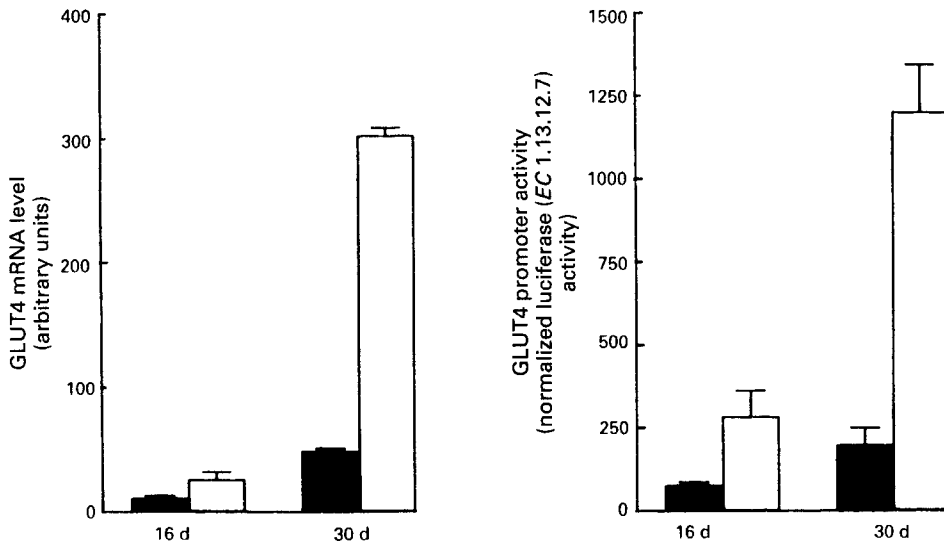


Fig. 4. GLUT4 mRNA and GLUT4 promoter activity in adipose cells from lean (■) and obese (□) Zucker rats. Values are means with their standard errors represented by vertical bars.

reported increased GLUT4 mRNA levels in white adipose tissue from young suckling and weaned obese Zucker rats. This suggests that the transcriptional step could be involved in the *fa*-dependent increase in GLUT4 gene expression. To address this question, we have examined the activity of the rat GLUT4 promoter fused to luciferase (*EC* 1.13.12.7) in transiently-transfected rat adipose cells (Hainault *et al.* 1995). As shown in Fig. 4, the transcriptional activity of the GLUT4 promoter is several-fold higher in fat cells from obese rats relative to lean rats, both at 16 and 30 d of age. Furthermore, the increase in GLUT4 promoter activity in obese *v.* lean parallels the increase in GLUT4 mRNA, strongly suggesting that the fatty genotype regulates GLUT4 gene expression at the transcriptional level. Interestingly, it has been recently shown in our laboratory that fatty acid synthase (*EC* 2.3.1.85; FAS), a key enzyme in lipogenesis from glucose, is also overexpressed in adipose cells from young obese rats, due to an increase in transcription rate and promoter activity (Guichard *et al.* 1992; Rolland *et al.* 1995). These findings suggest that the *fa* gene affects the activity of transcription factors in adipose cells, the nature of which remains to be determined. By activating genes crucial for triacylglycerol synthesis such as GLUT4 and FAS, these *fa*-dependent transacting factors have the potential to account for the development of obesity.

DYSREGULATION OF GLUT4 AND GLUT5 IN ADIPOSE TISSUE OF OBESE PATIENTS WITH NON-INSULIN-DEPENDENT DIABETES MELLITUS

A common pathology associated with obesity is insulin resistance and NIDDM. Recent studies have shown that GLUT4 expression is decreased in adipose cells from obese human subjects, a defect which is more profound in obese NIDDM patients (for references, see Bastar *et al.* 1995). However, although skeletal muscle is thought to be a

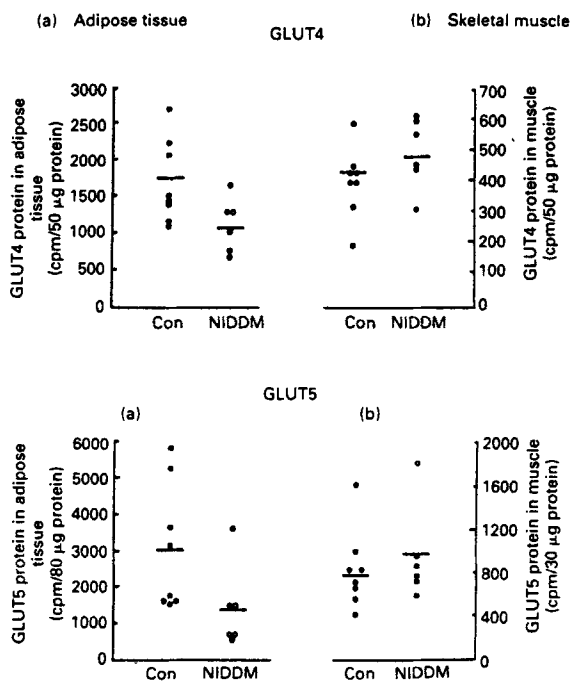


Fig. 5. GLUT4 and GLUT5 concentrations in (a) adipose cells and (b) muscle biopsies obtained from obese patients without (Con) or with non-insulin-dependent diabetes mellitus (NIDDM). cpm, Counts per min.

major site of insulin resistance in NIDDM, normal GLUT4 levels in muscle have been consistently observed (for references, see Bastar *et al.* 1995), suggesting that other factors, such as decreased GLUT4 activity, might be involved. As shown in Fig. 5, we have recently confirmed these observations using a protocol where GLUT4 concentrations were assessed in skeletal muscle and subcutaneous adipose tissue biopsies obtained from the same patient (Bastar *et al.* 1995). Furthermore, we observed a similar pattern of alteration for GLUT5, another isoform of transporter expressed in human adipose cells. These observations suggest that the expression of the two isoforms is regulated by common mechanisms. However, the specific role of GLUT5, a putative fructose transporter, in insulin-responsive tissues and its implication in NIDDM requires clarification.

CONCLUSION

These studies indicate that the expression of glucose transporter proteins in adipose cells is profoundly altered in obesity. Among the isoforms, GLUT4 is the most affected. In the early phase of the syndrome, GLUT4 is markedly overexpressed, a defect which would act as a 'metabolic pull' channelling glucose into triacylglycerols leading to increased fat cell size. Later on, GLUT4 levels decrease in conjunction with insulin resistance and eventually a NIDDM-like state. The molecular mechanisms involved in these dysregulations are yet to be determined. However, these studies emphasize that GLUT4 gene expression in adipose cells is a potential target for therapeutic agents in the treatment or prevention of obesity.

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