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## **The extracellular matrix and insulin resistance**

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# Trends in Endocrinology and Metabolism

## The Extracellular Matrix and Insulin Resistance

--Manuscript Draft--

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<b>Abstract:</b>	The extracellular matrix (ECM) is a highly dynamic compartment that undergoes remodeling as a result of injury and repair. Over the past decade, mounting evidence in humans and rodents suggest that ECM remodeling is associated with diet-induced insulin resistance in several metabolic tissues. Additionally, integrin receptors for the ECM have also been implicated in the regulation of insulin action. This review will address what is currently known about the ECM, integrins and insulin action in the muscle, liver and adipose tissue. Understanding how ECM remodeling and integrin signaling regulates insulin action may aid in the development of new therapeutic targets for the treatment of insulin resistance and type 2 diabetes.

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# The Extracellular Matrix and Insulin Resistance

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24 **Abstract:**

25 The extracellular matrix (ECM) is a highly dynamic compartment that undergoes remodeling as a  
26 result of injury and repair. Over the past decade, mounting evidence in humans and rodents suggest  
27 that ECM remodeling is associated with diet-induced insulin resistance in several metabolic  
28 tissues. Additionally, integrin receptors for the ECM have also been implicated in the regulation  
29 of insulin action. This review will address what is currently known about the ECM, integrins and  
30 insulin action in the muscle, liver and adipose tissue. Understanding how ECM remodeling and  
31 integrin signaling regulates insulin action may aid in the development of new therapeutic targets  
32 for the treatment of insulin resistance and type 2 diabetes.

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## 35 **Overview of the extracellular matrix and integrins**

36 The extracellular matrix (ECM) (Glossary) is composed of a diverse network of proteins and  
37 proteoglycans [1]. It provides a scaffold for cells and modulates biological processes including  
38 differentiation, cell migration, repair and development [2, 3]. The interaction between cells and  
39 the ECM is important for all organs. The ECM communicates with cells through transmembrane  
40 cell surface receptors called integrins [4]. Integrins bind the ECM and transduce signals through  
41 the plasma membrane to activate intracellular signaling. Integrins themselves lack kinase activity.  
42 Thus, they are reliant on scaffolding proteins and downstream kinases for signal transduction.  
43 Integrins signal through various proteins including focal adhesion kinase (FAK) and integrin-  
44 linked kinase (ILK) (**Box 1**). The detailed structure and function of integrins have been reviewed  
45 elsewhere [1, 4, 5].

46  
47 The ECM is a dynamic structure that remodels during times of injury and repair [6]. Pathological  
48 states are associated with ECM remodeling and alterations in integrin expression. In obese  
49 conditions, the expression of ECM proteins increases several-fold, while a shift appears to occur  
50 from low-density ECM proteins to more fibril-forming proteins. Several recent lines of evidence  
51 suggest that ECM remodeling and changes in integrin signaling in the diet-induced obese (DIO)  
52 state are associated with insulin resistance [7-16]. The potential mechanisms whereby this occurs  
53 are represented in **Figure 1**. Herein we discuss recent findings related to the emerging link between  
54 ECM remodeling, integrin signaling and insulin resistance in the skeletal muscle, liver, and  
55 adipose tissue.

56

57

## 58 **The Skeletal Muscle**

### 59 *Mechanisms of High Fat Diet-induced ECM Remodeling in the Skeletal Muscle*

60 Inflammation and elevated transforming growth factor (TGF)  $\beta$  signaling are associated with  
61 muscle ECM remodeling, in obese mice and humans [17]. Mice fed a high fat diet (HFD) exhibit  
62 increased infiltration of pro-inflammatory M1-activated (CD11c<sup>+</sup>) macrophages in muscle [18].  
63 Additionally, CD68<sup>+</sup> macrophages are elevated in obese individuals [19]. The association between  
64 ECM remodeling and inflammation was further shown in a study by Kang et al. [9]. In this study,  
65 20 weeks of HF feeding in mice led to increased muscle collagen content associated with increased  
66 gene expression of the pro-inflammatory marker tumor necrosis factor (TNF $\alpha$ ) and the  
67 macrophage marker F4/80. Importantly, gene expression for these inflammatory markers was  
68 diminished in mouse models of improved insulin sensitivity and decreased muscle collagen  
69 deposition. It is possible that increased recruitment of pro-inflammatory macrophages may lead to  
70 ECM remodeling via TGF $\beta$ -mediated Smad activation [20]. Smad3 activation is elevated in  
71 skeletal muscle biopsies of obese individuals compared to lean controls [17]. Collectively, this  
72 suggests that ECM remodeling in obese skeletal muscle occurs as a result of increased  
73 inflammation.

74

### 75 *The Skeletal Muscle ECM and Glucose Metabolism*

76 Insulin resistant muscle in obese and type 2 diabetic (T2D) humans is characterized by increased  
77 collagen deposition [7, 8]. Rapid weight gain in healthy young males resulted in impaired insulin  
78 sensitivity and the up-regulation of several muscle ECM genes [21]. There was no evidence of  
79 local adipose tissue or systemic inflammation despite weight gain, suggesting a key role for muscle  
80 ECM in the regulation of glucose homeostasis rather than secondary effects due to adipose tissue

81 inflammation.

82

83 Muscle collagen content is also increased in DIO, insulin resistant mice [9]. Studies by Kang et al.  
84 showed that increased collagen deposition in the DIO state is due to in part to decreased muscle  
85 matrix metalloproteinase 9 (MMP9) activity [9], and that the genetic deletion of MMP9 in mice  
86 increases collagen deposition in the muscle and exacerbates muscle insulin resistance in HF-fed  
87 mice [15].

88

89 Hyaluronan is an anionic, nonsulfated glycosaminoglycan. As a major component of the ECM,  
90 hyaluronan has multiple functions, including creating space between cells [22]. Serum hyaluronan  
91 is increased in T2D [23]. Insulin resistant animals have increased hyaluronan in muscles [16], aorta  
92 [24], and kidneys [25]. Elevated muscle hyaluronan levels are associated with muscle insulin  
93 resistance in the obese state. A reduction of muscle hyaluronan by intravenous injection of  
94 pegylated human recombinant hyaluronidase PH-20 (PEGPH20) results in a dose-dependent  
95 increase in glucose infusion rate and muscle glucose uptake during a hyperinsulinemic-euglycemic  
96 clamp [16]. This study showed for the first time that whole-body depletion of an ECM  
97 polysaccharide rescues insulin sensitivity in C57BL/6J HF-fed mice.

98

99 There are several hypotheses as to how increased muscle ECM in the HF-fed state contributes to  
100 insulin resistance, which may co-exist. A first hypothesis, the ECM is a physical barrier to both  
101 glucose and insulin diffusion. Proteins buildup in the interstitial space and this impedes substrate  
102 delivery to the muscle by increasing diffusion distance. A second hypothesis is that increases in  
103 muscle ECM impair neo-vascular growth and vascular function. The ECM is in close contact with

104 the endothelium. Blood flow and capillary recruitment are critical for proper glucose and insulin  
105 delivery to the muscle. Vascular dysfunction and capillary rarefaction (reduced capillary density)  
106 have long been implicated in the development of muscle insulin resistance and T2D [26]. Reduced  
107 blood flow to the muscle is correlated with insulin resistance, and conversely, the number of  
108 muscle capillaries is positively related to peripheral insulin action [27, 28]. Additionally, three  
109 weeks of treatment with the hormone relaxin, improved muscle insulin action through effects on  
110 the vasculature [29]. Kang and colleagues have provided consistent evidence that increased muscle  
111 capillaries are associated with improved muscle insulin action in HF-fed mice [9, 15]. This was  
112 evident in several mouse models, including the muscle-specific mitochondrial targeted catalase  
113 transgenic mice [9], chronic sildenafil-treated mice [9] and hyaluronidase-treated mice [16]. In  
114 contrast, decreased muscle capillaries are associated with exacerbated muscle insulin resistance in  
115 the global MMP9 knockout mouse [15]. It is important to consider that the first and second  
116 hypotheses are inextricably linked, as a decrease in capillarity will increase spatial barriers and  
117 diffusion distance for hormones and nutrients. Collectively, these data strongly suggest endothelial  
118 dysfunction and muscle capillary rarefaction are potential mechanisms by which ECM remodeling  
119 mediates muscle insulin resistance. Finally, the ECM may signal directly through muscle integrins  
120 to modulate insulin action (Figure 2). This is discussed in detail below.

121

### 122 *Integrins and Skeletal Muscle Insulin Resistance*

123 Skeletal muscle expresses seven integrin  $\alpha$  subunits ( $\alpha 1$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$ ,  $\alpha 6$ ,  $\alpha 7$ , and  $\alpha v$ ), and are all  
124 associated with the  $\beta 1$  integrin subunit [30]. Remarkably, few studies have addressed the role of  
125 integrin signaling in the muscle with respect to muscle insulin resistance *in vivo*. The muscle-  
126 specific deletion of integrin  $\beta 1$  in chow-fed mice results in decreased whole-body insulin



127 sensitivity, and decreased insulin-stimulated muscle glucose uptake during a hyperinsulinemic-  
128 euglycemic clamp [31]. Notably, the loss of skeletal muscle  $\beta 1$  has no effect on liver or adipose  
129 tissue glucose metabolism. The decrease in insulin-stimulated muscle glucose uptake was  
130 associated with decreased muscle glycogen synthesis and decreased Akt S473 phosphorylation.  
131 Moreover, the whole-body deletion of integrin  $\alpha 2$  in obese, HF-fed mice, partially reverses diet-  
132 induced muscle insulin resistance, as evidenced by increased insulin-stimulated muscle glucose  
133 uptake during a hyperinsulinemic-euglycemic clamp, and increased insulin signaling [9] (Figure  
134 3). These data suggest that integrin signaling might be a mechanistic link between the muscle ECM  
135 and insulin resistance.

136

137 The downstream integrin signaling molecule, FAK, has been implicated in the regulation of insulin  
138 action in the muscle [32-34]. FAK tyrosine phosphorylation is decreased in muscle from HF-fed  
139 rats [32]. The *in vivo* siRNA-mediated knockdown of FAK results in hyperglycemia,  
140 hyperinsulinemia, impaired glucose tolerance and decreased insulin action in chow-fed mice [34].  
141 The overexpression of FAK in C2C12 mouse myoblasts increases insulin-stimulated glucose  
142 uptake [35]. Conversely, C2C12 cells transfected with siRNA against FAK exhibit decreased  
143 insulin-stimulated glucose uptake [32]. L6 myocytes transfected with antisense FAK display  
144 decreased insulin signaling associated with decreased insulin-stimulated glucose uptake, decreased  
145 glycogen synthesis and impaired Glut4 translocation [33]. Collectively, these studies suggest that  
146 integrins mediate muscle glucose metabolism via their effects on both vascularization and glucose  
147 transport through Glut4.

148

149 No information exists about the role of ILK in the regulation of muscle glucose homeostasis. Mice  
150 with a muscle-specific deletion of ILK have been generated and are viable [36]. Considering the  
151 interaction of ILK with several known insulin signaling molecules such as Akt and GSK-3 $\beta$ , it is  
152 highly possible that ILK modulates muscle insulin action. Future studies should be designed to  
153 determine whether ILK regulates muscle insulin action in the DIO mouse model.

154

#### 155 *ECM remodeling and mechano-signal transduction*

156 ECM remodeling is also reflected by alterations in mechano-signal transduction to the nucleus and  
157 mitochondria [37, 38]. This may be a consequence of disturbances in actin and intermediate  
158 filament organization and/or the sarcoglycan complex, a junction whereby the myofiber interacts  
159 with the ECM. The sarcoglycan complex is critical for both force and mechano-signal transduction  
160 to the nucleus and mitochondrion. Disturbances in this complex produce metabolic effects [39].  
161 Mice lacking the sarcoglycan complex in the muscle and adipose tissue demonstrate whole body  
162 insulin resistance attributed to impaired insulin-stimulated muscle glucose uptake [39].  
163 Additionally, changes in the ECM of insulin resistant human muscle are accompanied by  
164 decreased abundance of the key filament organizational proteins, actinin 2 and desmin. It is  
165 plausible that these alterations may impair the ability of the muscle to adapt to exercise via  
166 compromised mechano-signal transduction to the nucleus or mitochondria that, under normal  
167 conditions, would induce gene transcription in response to exercise. In support of the ECM  
168 modulating mitochondrial function in the skeletal muscle, there is evidence that alterations in the  
169 collagen VI composition of the matrix affect mitochondrial function [37, 38]. Insulin resistant  
170 muscle is characterized by alterations in exercise tolerance and mitochondrial function, thus this  
171 provides another route whereby the ECM may regulate muscle insulin action.

## 172 **The Liver**

### 173 *Mechanisms of HFD-induced ECM Remodeling in the Liver*

174 The liver ECM expands with over-nutrition. Mice fed a HFD display increased hepatic staining  
175 for  $\alpha$ -smooth muscle actin (SMA, a marker of stellate cell activation) and collagen, as well as  
176 increased collagen type I  $\alpha$ 1 gene expression [11]. Mice fed a HFD with high fructose water exhibit  
177 increased hepatic collagen type I  $\alpha$ 1 gene expression [10]. Moreover, Williams et al. recently  
178 demonstrated that mice fed a 60% HFD exhibit increased gene expression for collagen types I and  
179 III [12].

180

181 The specific process whereby ECM remodeling in the liver occurs in the presence of over-nutrition  
182 is undefined. However, one prevailing hypothesis is a “two hit” hypothesis [40]. The “first hit” is  
183 the accumulation of lipid metabolites. This leads to a series of events including lipotoxicity,  
184 oxidative stress, and inflammation that produce a “second hit”. The “second hit” promotes tissue  
185 injury and the activation of stellate cells. This process is initiated by autocrine and paracrine stimuli  
186 including inflammatory cytokines and growth factors such as TGF $\beta$  [41-43]. Increased TGF $\beta$   
187 signaling is associated with hepatic collagen synthesis [44]. Once activated, stellate cells deposit  
188 ECM proteins in the space of Disse as part of a wound healing response, resulting in changes in  
189 the ECM and fibrosis [45]. Although it has been widely proposed that stellate cells are the main  
190 contributor to ECM deposition in the liver, it is possible that other cell types are involved [46].  
191 The notion that stellate cells are the main contributor was based on *in vitro* studies performed in  
192 cell culture [47, 48]. However, several cell types in the intact liver are capable of ECM synthesis,  
193 including hepatocytes, endothelial cells, as well as stellate cells [2]. Considering that hepatocytes

194 comprise approximately 80% of the liver [49], it is feasible that they contribute to the hepatic  
195 ECM.

196

### 197 *The Liver ECM and Glucose Metabolism*

198 T2D in humans is associated with hepatic ECM remodeling [50, 51]. Patients with T2D exhibit  
199 increased staining for collagen type IV,  $\alpha$ -SMA and a tendency for increased laminin staining [50].

200 In a separate study, liver biopsies from diabetic patients showed increased perisinusoidal fibrosis,  
201 characterized by immunostaining for laminin in sinusoidal spaces, as well as collagen type IV and  
202  $\alpha$ -SMA in the space of Disse [51]. It is important to note that early markers of ECM remodeling  
203 occur in diabetic patients prior to more advanced fibrosis and cirrhosis.

204

205 It is evident that a diet high in fat is associated with insulin resistance and ECM remodeling in the  
206 liver. Bonner et al. [29] showed that three weeks of relaxin treatment in HF-fed mice, results in  
207 decreased hepatic collagen type III , and a subsequent improvement in hepatic insulin action. In  
208 light of this, it is important to note that only one study to date has demonstrated a causal link  
209 between ECM remodeling and insulin resistance [16]. Kang et al. showed that depletion of  
210 systemic hyaluronan via tail vein injection of a long-acting hyaluronidase reverses HFD-induced  
211 liver insulin resistance [16].

212

213 CD44, the main hyaluronan cell surface receptor, is associated with T2D, as shown by expression-  
214 based genome-wide association studies (GWAS) [52]. CD44 is ubiquitously expressed, and its  
215 expression level in liver is positively correlated with hepatic steatosis and insulin resistance, in  
216 obese humans and DIO mice [52, 53]. Kodama et al. reported that anti-CD44 antibody treatment

217 lowers glycemia, improves insulin sensitivity and hepatic steatosis in DIO mice [54]. It is  
218 important to note that CD44 can also interact with other ligands, such as osteopontin, collagens  
219 and MMPs. Therefore, it is unclear whether the phenotype of mice lacking functional CD44 is due  
220 to prevention of hyaluronan or osteopontin or both. Hence, the role of CD44 signaling in diet-  
221 induced insulin resistance remains unclear, and warrants future investigation. Collectively, these  
222 studies highlight the role of the liver ECM in the regulation of glucose homeostasis.

223

224 There are two existing hypotheses as to how the hepatic ECM contributes to changes in insulin  
225 action. The first is through cellular and microcirculatory changes as a result of diet-induced ECM  
226 remodeling. In the liver, it is reasonable to speculate that diet-induced ECM remodeling (i.e.  
227 sinusoidal capillarization) sensitizes the liver to further damage and may facilitate maladaptive  
228 changes in hepatic insulin action [55]. The liver is a major site of insulin clearance. It is estimated  
229 that 50% of insulin is extracted by the liver during the first pass, via a receptor-mediated process  
230 [56-58]. Hepatic insulin extraction from the circulation reflects the ability of the liver to adequately  
231 respond to an insulin stimulus. Patients with cirrhosis and chronic hepatitis display decreased  
232 hepatic insulin extraction, compared to normal subjects [59]. This decrease in insulin clearance  
233 can be attributed to either liver damage or shunting of the portal-systemic circulation [59]. An  
234 extension of this is impaired insulin action and ultimately insulin resistance. A second hypothesis  
235 is that, as in muscle, the ECM signals through integrins and this regulates insulin action.

236

### 237 *Integrins and Liver Insulin Resistance*

238 Six  $\alpha$  integrin subunits ( $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\alpha 5$  and  $\alpha 6$ ) are expressed in the liver, all of which are  
239 associated with the  $\beta 1$  integrin [60]. Of these six  $\alpha$  subunits, only two integrins have been shown

240 to be expressed on the hepatocyte: integrin  $\alpha 1\beta 1$  and  $\alpha 5\beta 1$ . Integrin  $\alpha 5\beta 1$  is a fibronectin receptor,  
241 and integrin  $\alpha 1\beta 1$  is a collagen binding integrin. The genetic whole body deletion of the integrin  
242  $\alpha 1$  subunit in mice exists and is viable [61]. Studies show that integrin  $\alpha 1\beta 1$  protects against the  
243 development of hepatic insulin resistance [9, 12] (Figure 4). Williams et al. demonstrated that  
244 integrin  $\alpha 1$  protein expression is upregulated in hepatocytes isolated from HF-fed mice, compared  
245 to chow-fed controls [12]. Thus, to determine whether this response protected against hepatic  
246 metabolic impairments in DIO mice, insulin sensitivity was determined in integrin  $\alpha 1$ -null mice  
247 and their wild-type littermates. This study showed that deletion of the integrin  $\alpha 1$  subunit results  
248 in severe hepatic insulin resistance in HF-fed mice and decreased hepatic insulin signaling. It is  
249 currently unknown whether some unidentified integrin  $\alpha 1$  subunit binding protein is modulating  
250 the observed protective effect. Moreover, the role of integrin  $\alpha 5\beta 1$  in hepatic insulin action *in vivo*  
251 has not been investigated. It is possible, in the integrin  $\alpha 1$  subunit null mice, that the severe hepatic  
252 insulin resistance is attributable to enhanced integrin  $\alpha 5\beta 1$  signaling. Future studies should be  
253 conducted to identify novel integrin  $\alpha 1$  subunit binding partners and/or to determine whether  
254 integrin  $\alpha 5\beta 1$  contributes to or protects against diet-induced hepatic insulin resistance.

255

256 FAK has been heavily implicated in the regulation of glucose homeostasis and insulin action in  
257 the liver [33, 34, 62]. FAK undergoes rapid tyrosine phosphorylation in livers from healthy rats  
258 upon insulin stimulation under euglycemic conditions *in vivo* [62]. This is consistent with a  
259 separate study showing that HepG2 cells transfected with mutant FAK constructs display  
260 decreased Akt Ser473 and GSK-3 Ser9 phosphorylation [63]. There was no difference in the  
261 insulin receptor phosphorylation or PI3K activity, upon insulin stimulation suggesting that FAK  
262 exerts its actions on insulin signaling downstream of the insulin receptor [64]. FAK tyrosine

263 phosphorylation is decreased in HF-fed mice [12]. The *in vivo* siRNA-mediated knockdown of  
264 FAK results in hyperglycemia, hyperinsulinemia, and impaired glucose tolerance in chow-fed  
265 mice [34]. Finally, *fa/fa* rats treated with a TNF- $\alpha$  neutralizing agent exhibited increased hepatic  
266 FAK phosphorylation associated with decreased hepatic glucose output during an insulin clamp  
267 [62, 65]. These studies suggest that decreased integrin signaling through FAK may facilitate the  
268 development of hepatic insulin resistance.

269

270 Little is known about the role of integrin-linked kinase (ILK) in the regulation of hepatic insulin  
271 action. However, several studies suggest that ILK modulates the activation of several key insulin  
272 signaling proteins, including Akt and GSK-3 $\beta$  [66-68]. The stimulation of hepatic stellate cells in  
273 rats using carbon tetrachloride (CCl<sub>4</sub>) resulted in increased ILK protein expression, associated  
274 with enhanced phosphorylation of Akt, while the inhibition of ILK by siRNA prevented this [66].  
275 In contrast, the phosphorylation of Akt Ser473 is not affected in mice with a hepatocyte-specific  
276 deletion of ILK [67], and ILK-deficient cells are capable of phosphorylating Akt at both Thr308  
277 and Ser473 upon insulin stimulation, similar to control cells [68]. Thus, the role of ILK in the  
278 regulation of Akt and GSK-3 phosphorylation is currently unresolved and more studies are  
279 necessary to address whether ILK mediates insulin signaling *in vivo*. The regulation of hepatic  
280 insulin action by integrins is multifaceted, and more studies are necessary to determine the actions  
281 of each integrin and integrin signaling molecule on hepatic insulin action, to aid in the  
282 determination of future therapeutics.

283

284

285

## 286 **The Adipose Tissue**

287

### 288 *Mechanisms of High Fat Diet-induced ECM Remodeling in Adipose Tissue*

289 The adipose tissue responds dynamically to nutrient excess through adipocyte hypertrophy and  
290 hyperplasia [69]. This is followed by increased production of pro-inflammatory adipokines,  
291 immune cell infiltration and ECM remodeling. Excessive collagen deposition has been observed  
292 in the adipose tissue of various models of overnutrition [13, 70, 71]. Collagen VI is a highly  
293 enriched ECM protein in adipose tissue and its expression is increased in obese humans [70].  
294 Additionally, collagen gene expression (types I, III, V, and VI) is increased in adipose tissue from  
295 obese leptin receptor deficient *db/db* mice, and this is further exacerbated when the mice are fed a  
296 HFD [13]. Collectively, this suggests that ECM remodeling is a characteristic of obese adipose  
297 tissue.

298

299 Hypoxia and inflammation are stimuli for ECM remodeling during adipose tissue expansion. HF  
300 feeding in rodents leads to the doubling of fat cell area accompanied by local hypoxia [72].  
301 Hypoxia in obese adipose tissue occurs as the local vasculature fails to expand appropriately to  
302 meet the demands of increased fat mass. In support of this, gene expression of vascular endothelial  
303 growth factor (VEGF)-A and vessel density are decreased in the adipose tissue of *ob/ob* mice [72].  
304 Hypoxia then leads to HIF1 $\alpha$  activation and the secretion of pro-inflammatory cytokines from  
305 adipocytes [72, 73]. The combination of hypoxia and inflammation culminates in the pathological  
306 expansion of adipose ECM as adipocytes and recruited macrophages express and secrete collagens  
307 [70, 71, 74]. The mechanisms underlying ECM remodeling in adipose tissue have been reviewed  
308 in detail elsewhere [69, 75].



309 *The Adipose Tissue ECM and Glucose Metabolism*

310 One hallmark of metabolically dysfunctional adipose tissue is the pathological accumulation of  
311 ECM proteins. Increased collagen deposition is a physical barrier for adipocyte expansion during  
312 the development of obesity and this promotes the shunting of lipids into other tissues. A role for  
313 collagen in the obese, metabolically impaired adipose tissue was recently established [71]. The  
314 deletion of collagen VI in *ob/ob* mice results in un-impeded adipocyte expansion, improved  
315 glucose tolerance and insulin signaling. Additionally, the overexpression of the  $\alpha 3$  chain of  
316 collagen VI (endotrophin) in mice stimulates deposition of other collagen types, mainly collagen  
317 I, III and VI and insulin resistance in the presence of a HFD [76].

318

319 Thrombospondin 1 (THBS1) is a large adhesive ECM glycoprotein expressed predominantly in  
320 visceral adipose tissue and its expression is elevated in insulin-resistant, obese humans [19]. In  
321 mice, HF feeding acutely induces adipose tissue *Thbs1* expression and increases circulating  
322 THBS1 levels [14]. Genetic deletion of *Thbs1* in mice protects against HFD induced adipose tissue  
323 inflammation and insulin resistance [14]. Circulating THBS1 may also induce fibrosis in skeletal  
324 muscle and induce insulin resistance [14]. This is evident as *Thbs1*-null skeletal muscle are  
325 protected from HFD-induced collagen deposition and insulin resistance. Moreover, expression of  
326 the pro-inflammatory ECM glycoprotein, tenascin C, is also upregulated in the adipose tissue of  
327 obese mice and humans [77] and may contribute to insulin resistance.

328

329 The composition of ECM reflects a balance between matrix synthesis and degradation. Adipose  
330 tissue remodeling is altered in the obese state by ECM proteolysis via the fibrinolytic systems and  
331 matrix metalloproteinases (MMPs) [69]. MMPs are a family of zinc dependent proteinases

332 responsible for the degradation of ECM proteins [78]. MMP dysregulation has been implicated in  
333 the pathophysiology of obesity and diabetes. Plasma MMP2 and MMP9 concentrations are  
334 increased in obese [79] and diabetic [80, 81] individuals. Additionally, gene expression of adipose  
335 MMP9 correlates positively with altered HOMA-IR index in morbidly obese individuals [82].

336

337 MMPs are regulated by tissue inhibitors of metalloproteinases (TIMPs), which comprise a family  
338 of four protease inhibitors: TIMP1, TIMP2, TIMP3 and TIMP4 [83]. Circulating TIMP-1 and  
339 TIMP-2 are increased in patients with metabolic syndrome and diabetes [81]. Overexpression of  
340 TIMP1 in pancreatic  $\beta$ -cells protects mice from streptozotocin-induced  $\beta$ -cell death and diabetes  
341 [84]. Likewise, genetic deletion of TIMP2 results in obesity and glucose intolerance in chow-fed  
342 mice that is further exacerbated in HF-fed mice [85]. TIMP3 is reduced in the adipose tissue of  
343 experimental models of obesity and insulin resistance [86]. Genetic deletion of TIMP3 in mice  
344 promotes adipose tissue inflammation [87] and TIMP3 overexpression in macrophages protects  
345 against adipose tissue inflammation and insulin resistance [88]. These data suggest that increased  
346 tissue TIMPs are protective from insulin resistance. This is paradoxical as TIMPs inhibit MMP  
347 activities. Thus in-depth investigations into the role of MMPs and TIMPs in obesity and insulin  
348 resistance should be a fruitful future direction for research.

349

### 350 **Concluding remarks and future perspectives**

351 The regulation of insulin action in the DIO state is complex. Many questions remain as to how  
352 metabolic disease develops and persists over time. The ECM and integrins are emerging as critical  
353 regulators of insulin action in the muscle, liver and adipose tissue. Until recently, few studies had  
354 addressed the contribution of the extracellular compartment to the regulation of glucose

355 metabolism. The observation that ECM remodeling occurs in both human and rodent models of  
356 insulin resistance and T2D was a great step forward in understanding this previously  
357 uncharacterized portion of insulin sensitive tissues. ECM remodeling in the obese state has been  
358 attributed to increased inflammation and the subsequent up regulation of pro-fibrotic signaling  
359 molecules including TGF $\beta$ . It is currently unknown how the ECM regulates insulin action;  
360 however several hypotheses exist to explain this phenomenon. First, ECM remodeling generates a  
361 mechanical barrier for (a) glucose and insulin transport in the muscle and liver and (b) adipocyte  
362 hypertrophy in the adipose tissue under conditions of overnutrition. Second, changes in the  
363 composition of the ECM results in downstream alterations in integrin signaling that culminate in  
364 impaired insulin action. In light of these hypotheses, several important outstanding questions  
365 endure (**Box 2**). Future studies that seek to determine the mechanisms underlying diet-induced  
366 ECM remodeling and the mechanistic link between ECM remodeling, integrin signaling and  
367 insulin action in metabolic tissues are vital to advancing this great new line of investigation. In  
368 conclusion, the ECM and integrins are important regulators of insulin action and represent novel  
369 therapeutic targets to treat the underlying insulin resistance associated with T2D.

370

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607

## 608 **Figure Legends**

609 **Figure 1:** A link between extracellular matrix remodeling and insulin resistance.

610 A diet high in fat generates a state of chronic inflammation. This inflammatory response leads to  
611 increased ECM synthesis and decreased ECM degradation, resulting in increased deposition and  
612 remodeling of ECM. Increased levels of ECM lead to increased physical barriers for insulin and  
613 glucose transport, decreased vascular insulin delivery and decreased insulin signaling. The  
614 combination of all of these factors then culminates in insulin resistance.

615

616 **Figure 2:** The role of integrin  $\alpha 2\beta 1$  diet-induced muscle insulin resistance.

617 In the HF-fed state, capillary density and endothelial function are impaired. This results in  
618 decreased potential for glucose and insulin transport into the interstitial space despite  
619 hyperglycemia and hyperinsulinemia. Moreover, increased ECM deposition in the interstitial  
620 space also provides a physical barrier to glucose and insulin transport to the myocyte. Insulin  
621 signaling within the myocyte is impaired and this may be attributed to increased integrin  $\alpha 2\beta 1$   
622 signaling as a consequence of increased deposition of the ECM. This results in impaired Glut4  
623 translocation and decreased glucose transport into the myocyte. In contrast, the genetic deletion of  
624 the integrin  $\alpha 2$  subunit results in improved insulin-stimulated muscle glucose uptake.

625

626 **Figure 3:** The role of integrin  $\alpha 1\beta 1$  in diet-induced hepatic insulin resistance.

627 In the HF-fed state, sinusoidal capillarization occurs and this, in addition to increased ECM  
628 buildup in the space of Disse, results in decreased insulin transport to the hepatocyte despite  
629 hyperinsulinemia. Protein expression of the integrin  $\alpha 1$  subunit is increased and this leads to  
630 increased  $\alpha 1\beta 1$  cell signaling. Upon insulin stimulation, the combination of both insulin and  
631 integrin  $\alpha 1\beta 1$  signaling results in some insulin signaling and the partial suppression of hepatic  
632 glucose output. In contrast, the genetic deletion of the integrin  $\alpha 1$  subunit results in severe hepatic  
633 insulin resistance and no insulin-mediated suppression of hepatic glucose output. This is attributed  
634 to decreased insulin signaling. It is possible that this effect is mediated by integrin  $\alpha 5\beta 1$ , the only  
635 other known integrin expressed on the hepatocyte, however this is currently unknown.

636

637 **Figure 4:** Proposed model whereby integrins regulate insulin action.

638 In the presence of insulin, integrin signaling through both integrin linked kinase (ILK) and focal  
639 adhesion kinase (FAK) promotes insulin action. Canonical insulin signaling occurs, however it is  
640 possible that other mechanisms exist whereby insulin exerts its actions within the cell. Several  
641 studies show that FAK is an important regulator of insulin action in both the muscle and liver. Less  
642 is known about ILK. However, Nck2 is an adaptor protein shared by both the insulin receptor and  
643 ILK. This suggests that there may be a physical link between the insulin receptor and integrins  
644 through Nck2 and ILK, allowing the centralization of signaling through this complex. Akt, a  
645 critical insulin signaling molecule, is a known binding partner of ILK. Additionally, integrin  
646 signaling has been shown to modulate the assembly of the cytoskeleton and this may have effects  
647 on both mitochondrial function and insulin action.

648

649

650 **Text Box 1: Integrin signaling molecules**

651 *Focal adhesion kinase*

652 Focal adhesion kinase (FAK) is a cytoplasmic tyrosine kinase that localizes with integrin receptors  
653 at sites where cells attach to the ECM [89]. FAK undergoes rapid autophosphorylation at Tyr397  
654 upon integrin-mediated cell adhesion [90], and this is associated with increased catalytic activity.  
655 Additionally, FAK can be regulated by the growth factor receptors epidermal growth factor  
656 receptor (EGFR), fibroblast growth factor receptor (FGFR) and the insulin receptor [63, 91]. This  
657 results in the activation of several downstream signaling cascades including the MAPK and PI3K  
658 signaling pathways [63, 91]. In addition to its signaling properties, FAK is important for  
659 cytoskeletal stabilization and focal adhesion turnover [92].

660 *Integrin-linked kinase and insulin action*

661 Integrin-linked kinase (ILK) is a highly conserved intracellular scaffolding protein. It interacts  
662 with the  $\beta 1$ ,  $\beta 2$  and  $\beta 3$ -integrin cytoplasmic domains and numerous cytoskeleton-associated  
663 proteins. It is composed of three distinct domains: an N-terminus that contains five ankyrin repeats,  
664 a pleckstrin homology-like domain and a pseudokinase domain at the C-terminus. Considering  
665 that it is a scaffolding protein, it has been proposed that ILK modulates intracellular signaling  
666 through its ability to recruit a kinase or multiple kinases into a multiprotein complex. This complex  
667 then facilitates the activation of downstream signaling molecules upon insulin stimulation. The  
668 pseudokinase domain of ILK is an essential domain for the recruitment of adaptor proteins and/or  
669 signaling molecules including several proteins involved in insulin action such as PKB/Akt, PDK1  
670 and GSK-3 $\beta$ . Overexpression of ILK or insulin treatment results in increased GSK-3 and Akt  
671 phosphorylation [93]. Co-transfection of Akt with wild-type ILK in 293 cells resulted in an  
672 enhancement of phosphorylation of Akt Ser473 [93]. Several studies have shown that the ablation



673 of ILK results in decreased Akt Ser473 phosphorylation [94-96]. Moreover, ILK is connected to  
674 growth factor receptors through the adaptor protein Nck2 [97]. Therefore, although ILK lacks  
675 intrinsic kinase activity, it has been shown to regulate the activation of numerous intracellular  
676 growth factor signaling cascades [98-100].

677

## 678 **Box 2: Outstanding questions**

- 679 • What role does inflammation have in diet-induced ECM remodeling? Which components  
680 of the inflammatory process are involved? Are the inflammatory stimuli acting locally or  
681 are they systemic?
- 682 • When does ECM remodeling occur during a time-course of HF feeding in rodent models?  
683 How does this relate to insulin action?
- 684 • Does ECM remodeling lead to insulin resistance by generating a physical barrier for  
685 glucose and insulin transport?
- 686 • How do integrins regulate insulin action *in vivo*? What components of the integrin signaling  
687 cascade are important for insulin action?

688

## 689 **Glossary**

690 **Collagen:** the most abundant structural protein consisting of three  $\alpha$  polypeptide chains folded into  
691 a triple helix formation. Collagen proteins are divided into subgroups depending on their  
692 organization and/or molecular size that include the fibril forming collagens type I and III, the  
693 basement membrane associated collagen type IV and collagen type V, a minor ECM component.

694 **Cirrhosis:** late stage fibrosis of the liver as a result of different liver diseases and conditions such  
695 as hepatitis and chronic ethanol ingestion.

696 **Endothelial dysfunction:** deleterious alterations in endothelial physiology characterized by  
697 impaired endothelium-dependent vasodilation due to decreased availability of vasodilators such as  
698 NO and/or an increase in endothelium-derived contracting factors.

699 **Extracellular matrix (ECM):** the space outside the cell composed of a complex meshwork of  
700 different proteins, proteoglycans, glycoproteins, polysaccharides and other structural proteins.

701 **Glycosaminoglycans:** large linear polysaccharides containing repeating disaccharide units with  
702 an amino sugar (either GlcNAc or GalNAc) and an uronic acid. Five identified glycosaminoglycan  
703 chains exist: hyaluronan, dermatan, keratan, chondroitin and keratan.

704 **Homeostatic model assessment of insulin resistance (HOMA-IR):** method to assess insulin  
705 resistance and  $\beta$ -cell function, from basal (fasting) glucose and insulin or C-peptide concentrations.

706 **Hyaluronan:** an anionic, nonsulfated glycosaminoglycan. It is a major component of the ECM  
707 and has multiple functions, including creating space between cells and facilitating cell migration.

708 **Hyperinsulinemic-euglycemic clamp (insulin clamp):** the gold standard for assessing insulin  
709 action *in vivo*. During the insulin clamp, insulin is infused at a constant rate and glucose is infused  
710 at a variable rate to maintain euglycemia. The amount of glucose that is infused reflects the insulin  
711 sensitivity. The insulin clamp can be combined with tracer techniques to determine sites of insulin  
712 resistance.

713 **Interstitial space:** the narrow, fluid filled areas that surround the cells of a tissue.

714 **Myofibroblasts:** cells in a state between a fibroblast and a smooth muscle cell. Fibrogenic cells  
715 are not part of the normal tissue and are only present following cellular injury. Often characterized  
716 by the presence of ruffled membranes and a highly active endoplasmic reticulum.

717 **Nonalcoholic fatty liver disease (NAFLD):** also known as fatty liver disease, refers to the  
718 accumulation of excess lipids in liver cells that can induce inflammation and fibrosis.

719 **Oxidative stress:** the imbalance between the production of reactive oxygen species (ROS) and  
720 antioxidant defenses that may result in tissue damage.

721 **Relaxin:** a protein hormone that acts through two G-protein coupled receptors RXFP1 and RXFP2  
722 and has effects on the cardiovascular system. The vascular effects of relaxin include vasodilation  
723 and a decrease in systemic vascular resistance.

724 **Space of Disse:** The sinusoidal endothelium is separated from hepatocytes by the space of Disse  
725 where all metabolites from the bloodstream must pass through to reach the hepatocytes. The  
726 surface area of hepatocytes exposed to the space of Disse is greatly enhanced by the presence of  
727 microvilli. Under normal conditions, the space of Disse is filled with loosely assembled, low-  
728 density extracellular matrix (ECM) proteins.

729 **Stellate cells:** previously known as Ito cells, are quiescent vitamin A rich cells. Following liver  
730 injury, they transform into activated proliferative and fibrogenic myofibroblasts. This process is  
731 initiated by autocrine and paracrine stimuli including inflammatory cytokines and growth factors.

732

733

734

Figure 1  
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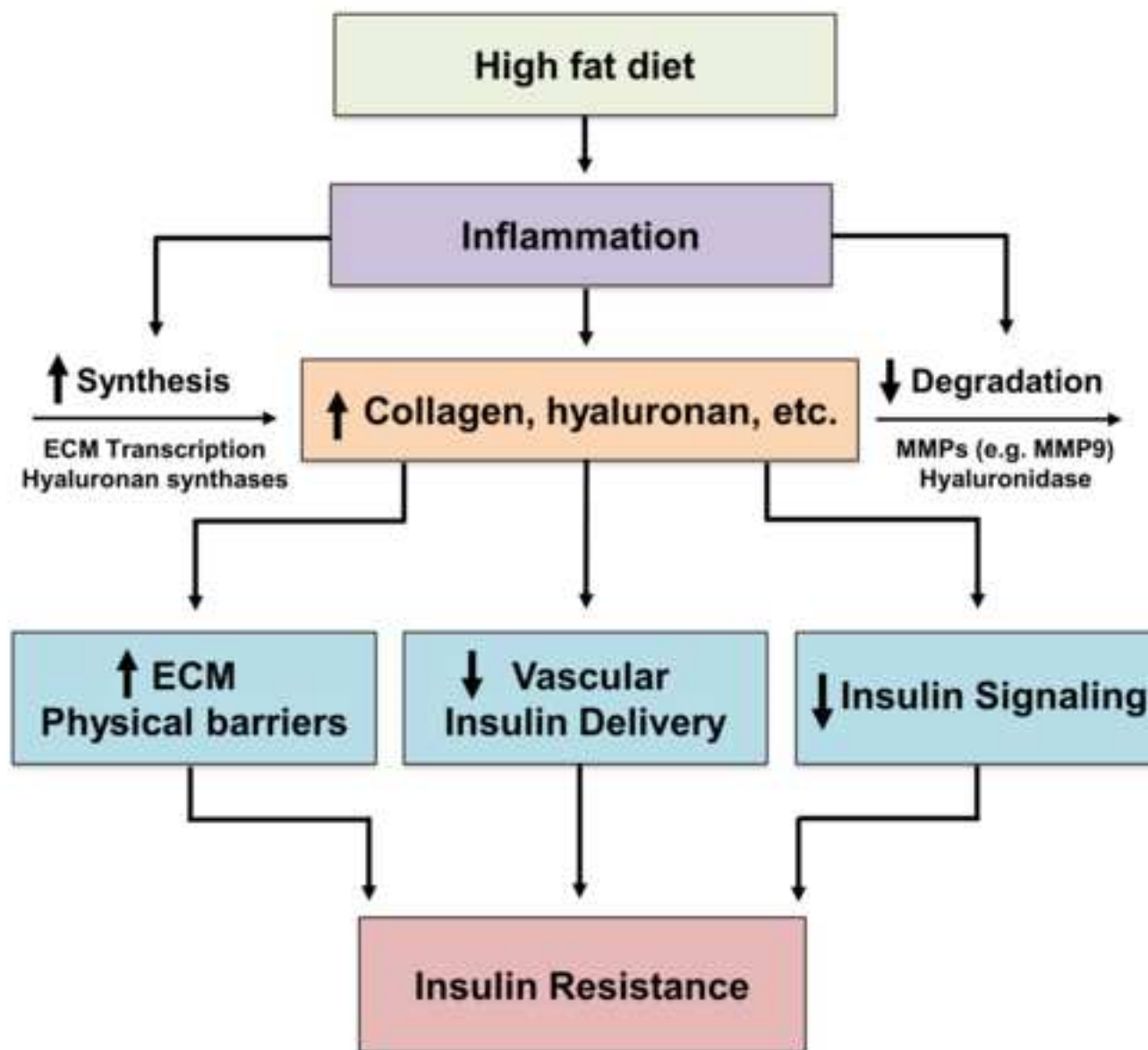
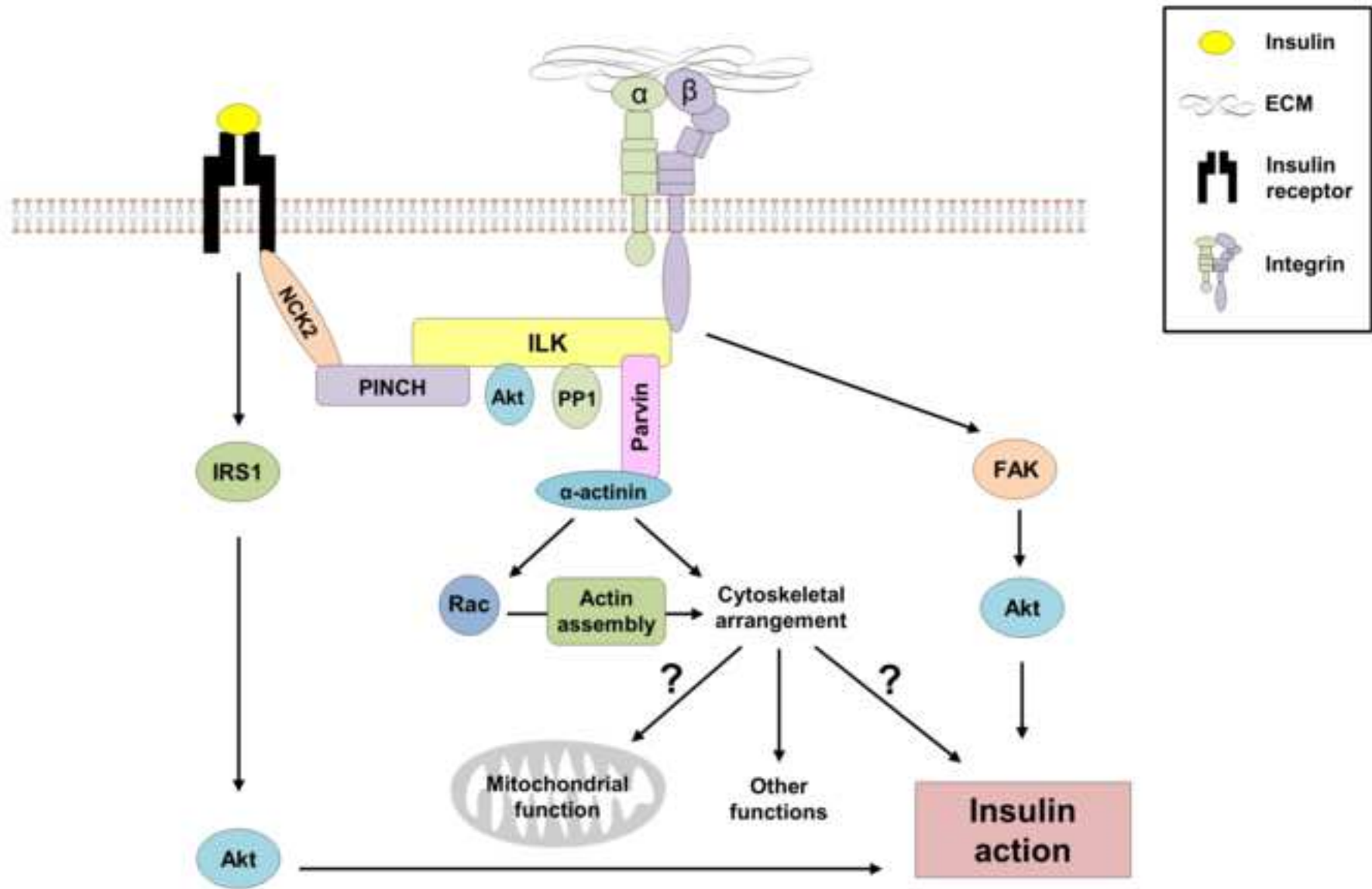
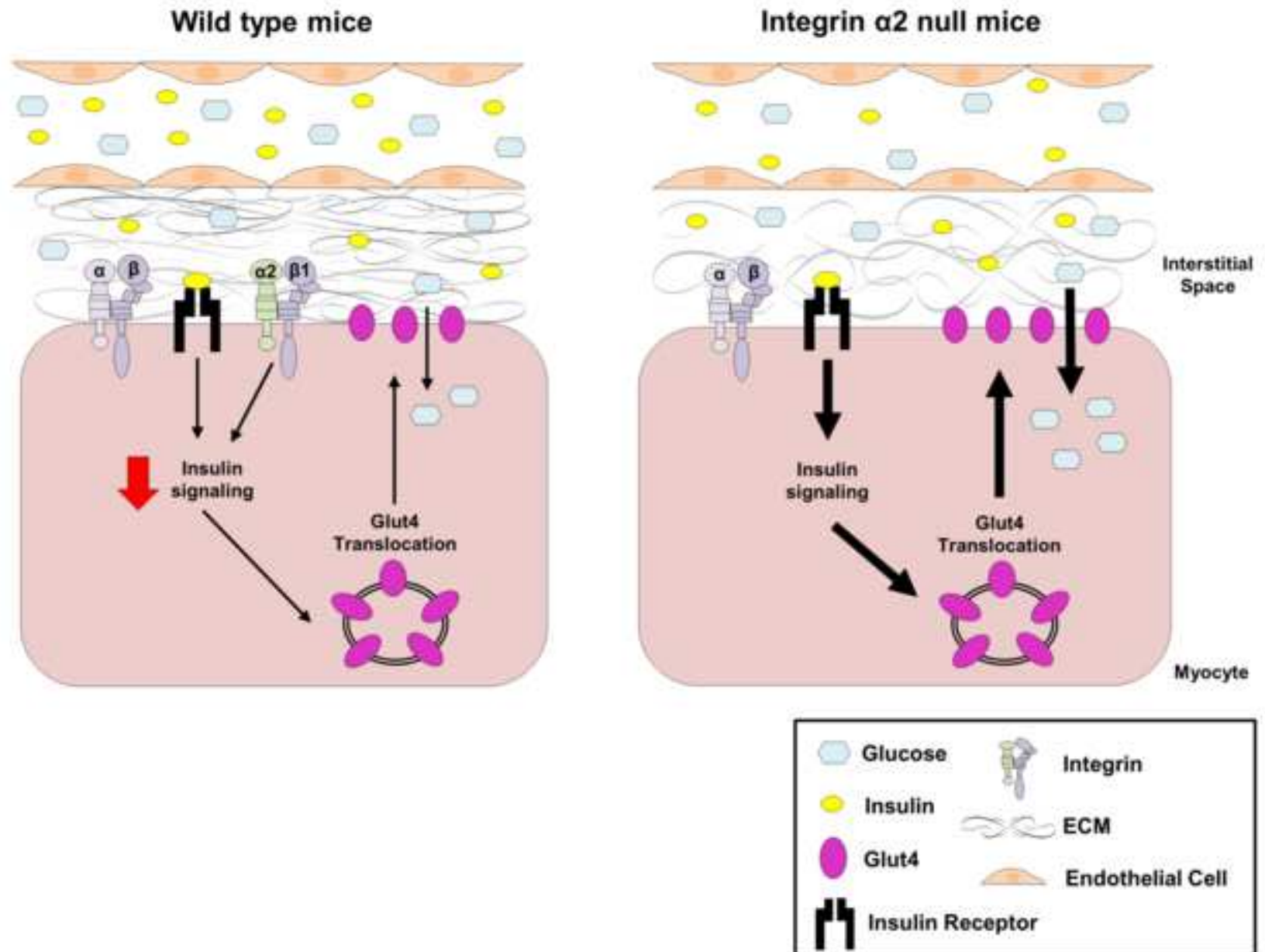


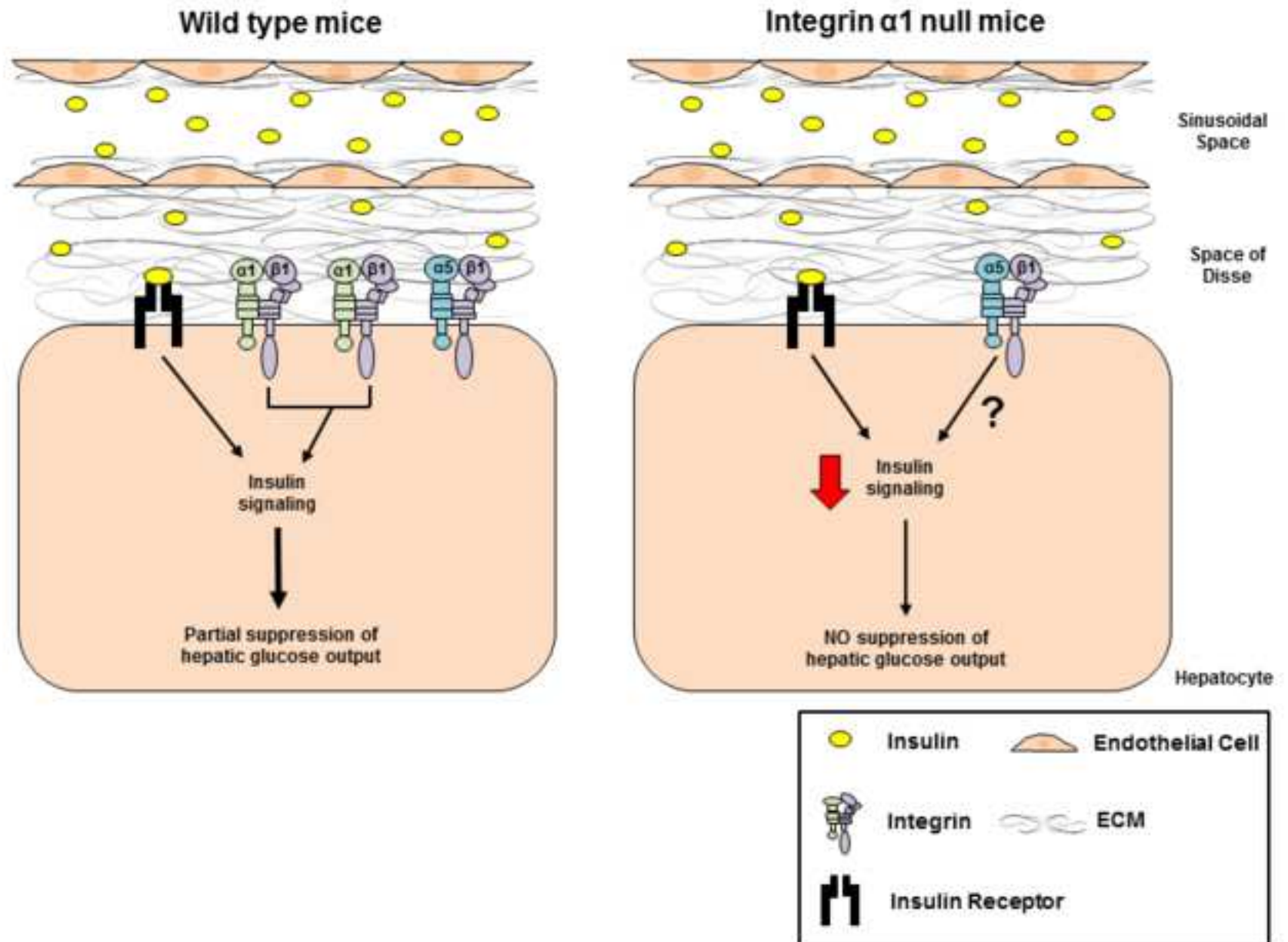
Figure 2  
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## Diet-induced muscle insulin resistance



## Diet-induced hepatic insulin resistance



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