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Abstract:	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.

1   **The Extracellular Matrix and Insulin Resistance**

2

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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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Overview of the extracellular matrix and integrins

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

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

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) β 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+) macrophages in muscle [18].

63 Additionally, CD68+ 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 (TNFα) 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 TGFβ-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 metallopeptidase 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 α subunits (α1, α3, α4, α5, α6, α7, and αv), and are all

124 associated with the β1 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 β1 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 β1 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 α2 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-3β, 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 α-smooth muscle actin (SMA, a marker of stellate cell activation) and collagen, as well as

176 increased collagen type I α1 gene expression [11]. Mice fed a HFD with high fructose water exhibit

177 increased hepatic collagen type I α1 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 TGFβ [41-43]. Increased TGFβ

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, α-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   α-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 α integrin subunits (α1, α2, α3, α4, α5 and α6) are expressed in the liver, all of which are

239 associated with the β1 integrin [60]. Of these six α subunits, only two integrins have been shown

240   to be expressed on the hepatocyte: integrin α1β1 and α5β1. Integrin α5β1 is a fibronectin receptor,

241   and integrin α1β1 is a collagen binding integrin. The genetic whole body deletion of the integrin

242   α1 subunit in mice exists and is viable [61]. Studies show that integrin α1β1 protects against the

243   development of hepatic insulin resistance [9, 12] (Figure 4). Williams et al. demonstrated that

244   integrin α1 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 α1-null mice

247   and their wild-type littermates. This study showed that deletion of the integrin α1 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 α1 subunit binding protein is modulating

250   the observed protective effect. Moreover, the role of integrin α5β1 in hepatic insulin action *in vivo*

251   has not been investigated. It is possible, in the integrin α1 subunit null mice, that the severe hepatic

252   insulin resistance is attributable to enhanced integrin α5β1 signaling. Future studies should be

253   conducted to identify novel integrin α1 subunit binding partners and/or to determine whether

254   integrin α5β1 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-α 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-3β [66-68]. The stimulation of hepatic stellate cells in

273 rats using carbon tetrachloride (CCl4) 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 HIF1α 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 α3 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, tenacin 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 β-cells protects mice from streptozotocin-induced β-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 TGFβ. 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.

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607

Figure Legends

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

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

Figure 2: The role of integrin α2β1 diet-induced muscle insulin resistance.

In the HF-fed state, capillary density and endothelial function are impaired. This results in decreased potential for glucose and insulin transport into the interstitial space despite hyperglycemia and hyperinsulinemia. Moreover, increased ECM deposition in the interstitial space also provides a physical barrier to glucose and insulin transport to the myocyte. Insulin signaling within the myocyte is impaired and this may be attributed to increased integrin α2β1 signaling as a consequence of increased deposition of the ECM. This results in impaired Glut4 translocation and decreased glucose transport into the myocyte. In contrast, the genetic deletion of the integrin α2 subunit results in improved insulin-stimulated muscle glucose uptake.

Figure 3: The role of integrin α1β1 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 α1 subunit is increased and this leads to

630   increased α1β1 cell signaling. Upon insulin stimulation, the combination of both insulin and

631   integrin α1β1 signaling results in some insulin signaling and the partial suppression of hepatic

632   glucose output. In contrast, the genetic deletion of the integrin α1 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 α5β1, 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 β1, β2 and β3-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-3β. 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 α 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.

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

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

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

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

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

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

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

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

Nonalcoholic fatty liver disease (NAFLD): also known as fatty liver disease, refers to the 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 transforms into activated proliferative and fibrogenic myofibroblasts. This process is

731   initiated by autocrine and paracrine stimuli including inflammatory cytokines and growth factors.
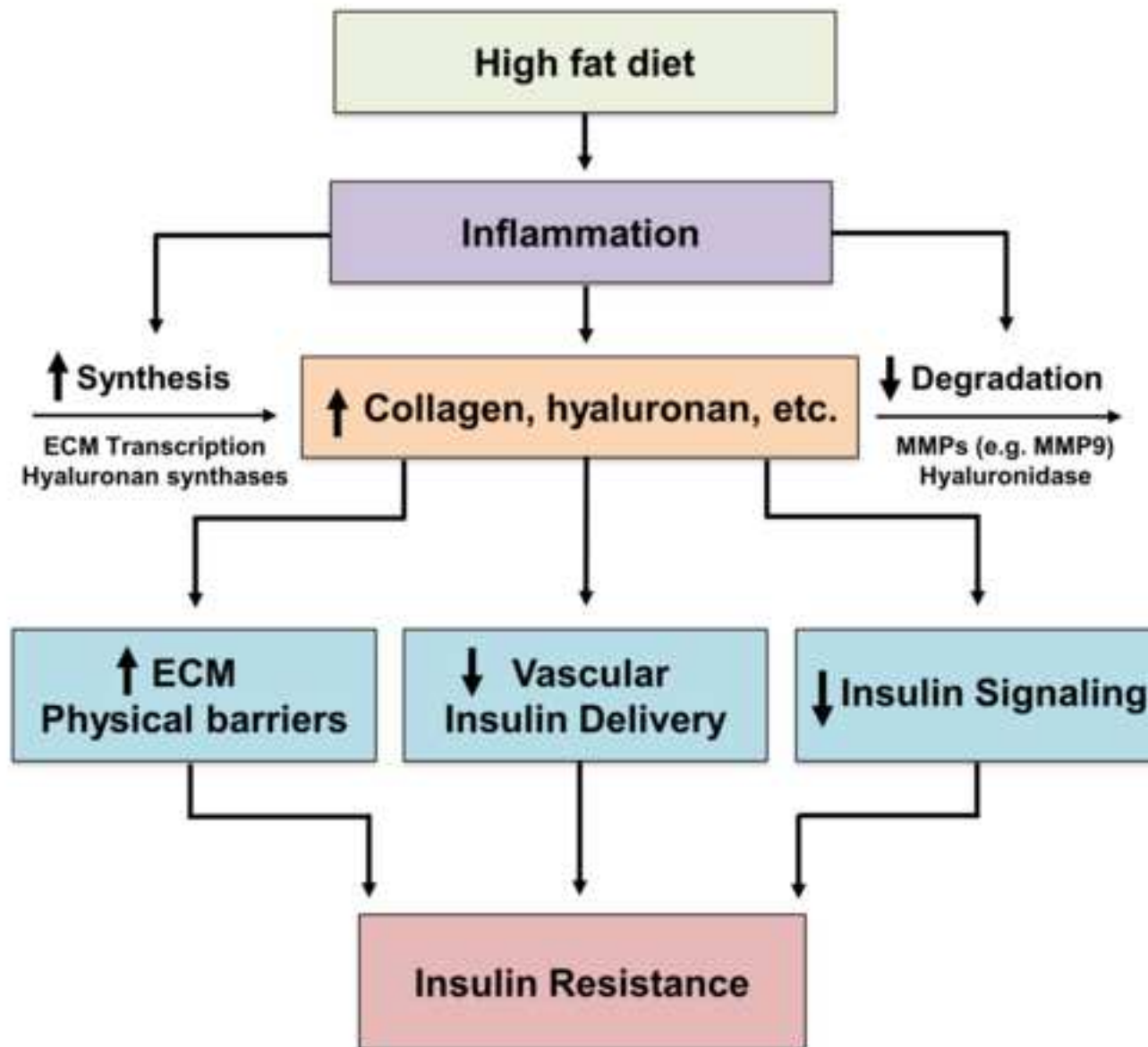
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Figure 1

Figure 2
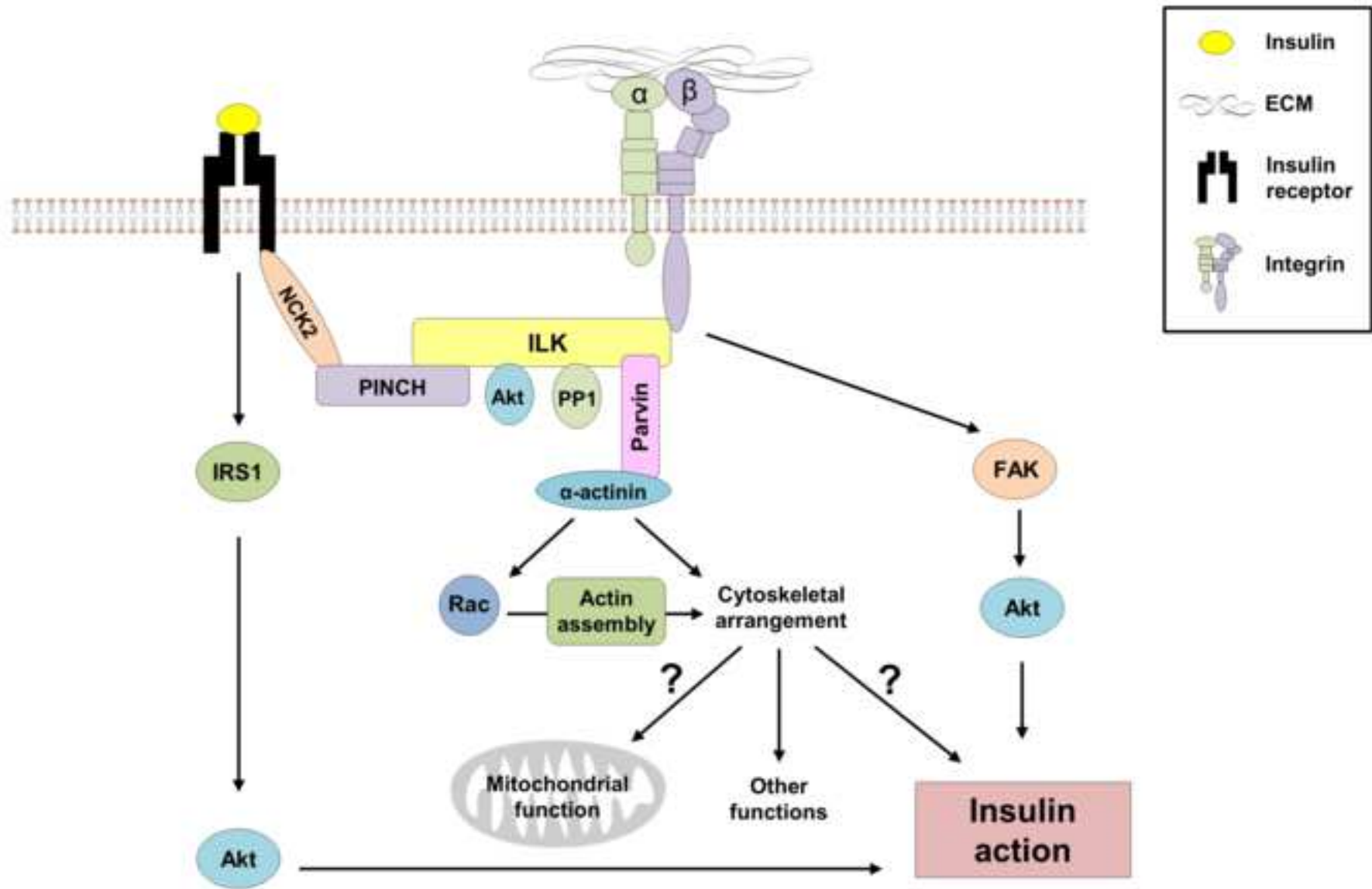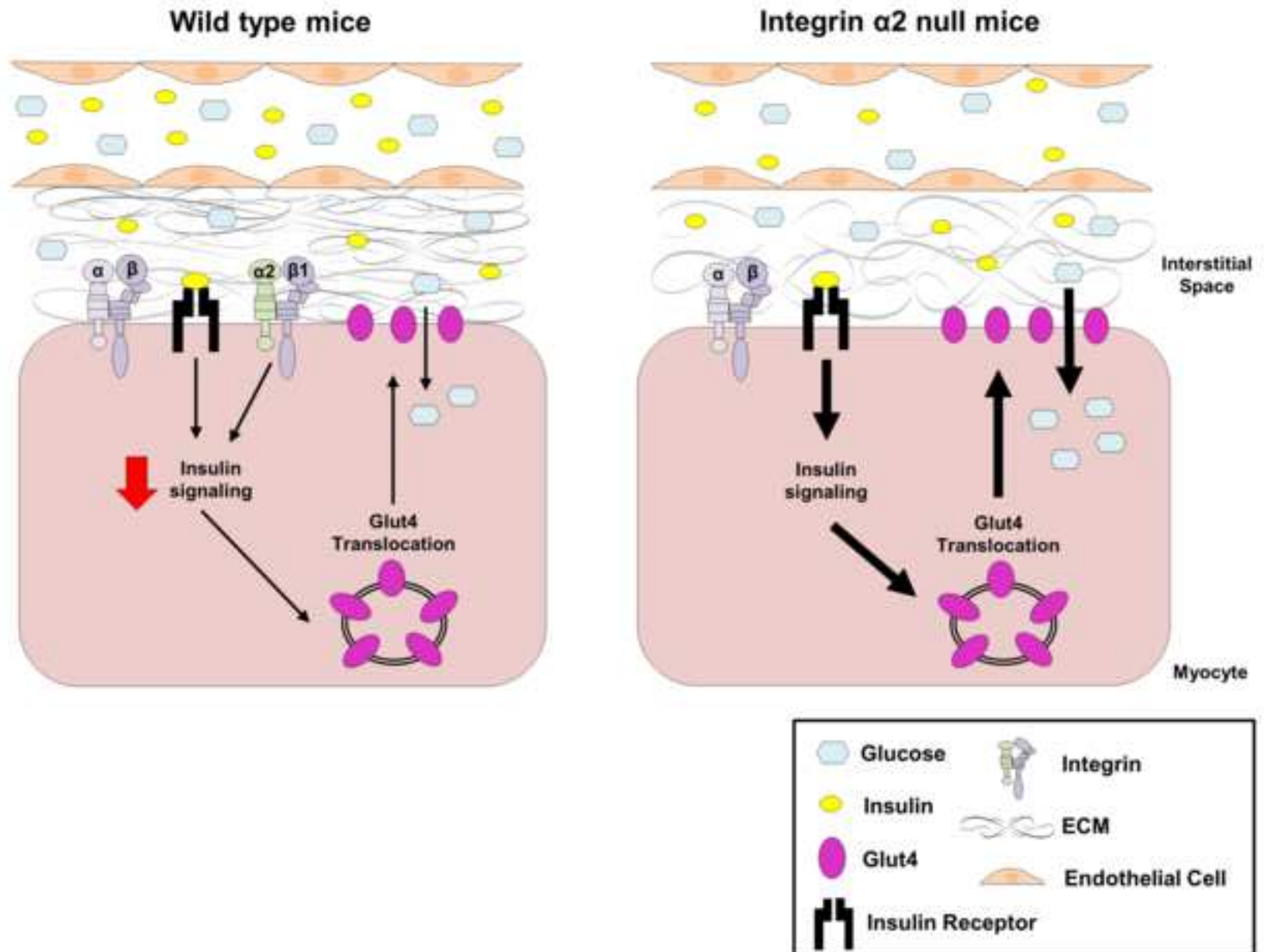Click here to download Figure: Figure 2.tif

Diet-induced muscle insulin resistance
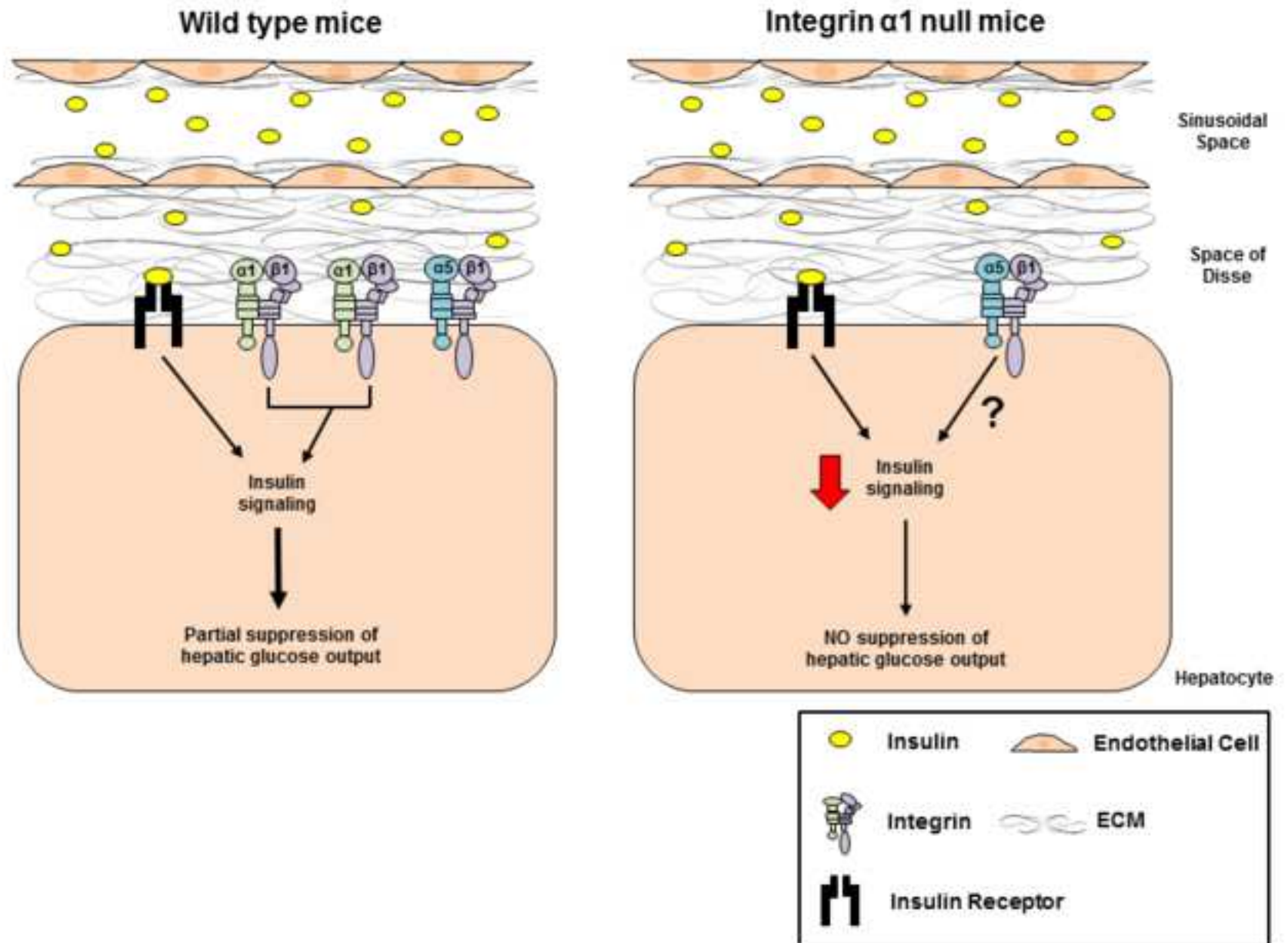
Figure 4
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Diet-induced hepatic insulin resistance

Wild type mice

Integrin α1 null mice

Sinusoidal Space

Space of Disse

α1 β1   α1 β1   α5 β1

α5 β1

Insulin signaling

Insulin signaling

?

Partial suppression of hepatic glucose output

NO suppression of hepatic glucose output

Hepatocyte

Insulin   Endothelial Cell

Integrin   ECM

Insulin Receptor