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1 **Tick-pathogen interactions: the metabolic perspective**

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3

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13

14 **Abstract**

15 The first tick genome published in 2016 provided an invaluable tool to study the molecular
16 basis of tick-pathogen interactions. Metabolism is a key element in host-pathogen
17 interactions. However, our knowledge of tick-pathogen metabolic interactions is very
18 limited. Recently, a Systems Biology approach using omics datasets has revealed that tick-
19 borne pathogen infection induces transcriptional reprogramming affecting several metabolic
20 pathways in ticks to facilitate infection, multiplication and transmission. Results suggest that
21 the response of tick cells to tick-borne pathogens is associated with tolerance to infection.
22 Here we review our current understanding of tick metabolism modulation by tick-borne
23 pathogens with focus in the model intracellular bacterium *Anaplasma phagocytophilum*.

24

25 **Keywords:** *Ixodes*; *Anaplasma phagocytophilum*; *Borrelia* spp.; metabolism; Tick-pathogen
26 interactions

27

28 **Tick-pathogen metabolic interactions at the center of the infectious storm**

29 Metabolism is key to cellular function [1,2]. In consequence, all major molecular pathways in
30 living cells are interconnected to and regulated by the availability and levels of metabolites
31 [1,2]. Recent studies in mammalian cells showed that interactions between intracellular
32 bacterial pathogens and the host cells can lead to physiological changes in both interacting
33 members [3]. The metabolic adaptations in mammalian cell-pathogen systems promote
34 proliferation or elimination of the pathogen within the host cells [3]. Tick-pathogen
35 interactions, however, involve a more complex array of outcomes which include conflict and
36 cooperation that ultimately benefit both ticks and pathogens [4]. This is not surprising
37 considering that in vector-borne pathogen systems the survival of the vector is essential for
38 the completion of the life cycle of the pathogen. In addition, tick-borne pathogen infection
39 increases tick performance in challenging environmental conditions [5,6]. Therefore, in some
40 cases, vector tolerance to pathogen infection is an advantageous life trait in arthropod
41 vectors [7].

42 Tick metabolism modulation by tick-borne pathogens is a result of coevolution and adaption
43 to a considerable number of tick and reservoir host species [4,8,9]. The *Ixodes scapularis*
44 genome, the first for a medically important chelicerate, is the only tick genome available so
45 far [10]. The sequencing of the *I. scapularis* genome was an essential step towards the
46 understanding of the molecular processes that support the parasitic lifestyle of the tick and
47 its success as a vector of multiple pathogens including bacteria, viruses, protozoa, and
48 helminths which constitute a growing burden for human and animal health worldwide [11].
49 This review focuses on recent research that provided insights on the finely tuned metabolic
50 changes that the model pathogen *Anaplasma phagocytophilum* induces in its tick vector *I.*
51 *scapularis*. For comparative purposes, tick metabolic changes induced by other pathogens
52 including mainly *Borrelia* spp., flaviviruses and fungi will be also included (Table 1). Results
53 suggest that tick-borne pathogens use tick metabolism as a hub to modulate tick physiology
54 and tick cell processes including immunity and apoptosis among others.

55

56 **Nutritional immunity and iron metabolism in ticks**

57 Iron is essential for most organisms because it serves as an electron donor and acceptor in
58 various metabolic processes. Therefore, iron holds a central position in host-pathogen
59 interactions [12]. While host evolved mechanisms to deprive pathogens from iron,
60 pathogens coevolved mechanisms to acquire host iron. Host mechanisms involved in limiting
61 pathogens access to iron were termed 'nutritional immunity' [12]. During feeding, ticks are
62 exposed to the large amounts of iron present in the blood meal. Excess iron can react with
63 H₂O₂ which yields hydroxyl radical, a potent biological oxidant [12]. Therefore, a balanced
64 regulation of iron levels is essential for ticks [13], and potentially, pathogen survival. Two
65 ferritin (FER) proteins, FER1 and FER2, are the primary iron storage and transporter,
66 respectively, in ticks [13]. In addition of controlling the production of reactive oxygen species
67 from iron, like in other model organism [12, 13], ferritins may also contribute to nutritional
68 immunity in ticks by depriving pathogens from essential iron supply. Interestingly,
69 *Escherichia coli* infection induces the expression of a *fer* gene in *Dermacentor variabilis* and
70 silencing of *fer* genes in *Haemaphysalis longicornis* decreases tick survival following a
71 challenge with *E. coli* [13].

72 The tick-borne pathogen *Ehrlichia ruminantium* sense iron levels in mammalian cells and
73 respond to iron starvation by upregulating virulence genes such as those involved in Type IV
74 Secretion System (T4SS), which in turn may participate in iron acquisition by the pathogen
75 [14]. A similar mechanism may be at play during pathogen infection in ticks. An iron-binding
76 protein expressed in the infective stage of *A. phagocytophilum* [15], and hypothesized to be
77 secreted by the T4SS, was found in tick salivary glands and tick cells infected with these
78 bacteria [16]. Bacterial iron-binding proteins may sequester iron to overcome nutritional
79 immunity in ticks. An interesting mechanism to scape nutritional immunity is that of *Borrelia*
80 *burgdorferi* which requires none or very low amounts of iron to survive [17]. In addition,
81 recent research provides evidence that *A. phagocytophilum* modulates tick heme
82 metabolism. Host hemoglobin is cleavage to heme by three sequential set of reactions
83 involving several hemoglobinolytic enzymes in ticks [18]. Due to the high redox potential of
84 heme, this molecule is toxic and in excess can be bacteriostatic [19]. Quantitative proteomics
85 analysis revealed that *A. phagocytophilum* modulates the levels of hemoglobinolytic
86 enzymes which affects the levels of host hemoglobin in tick midguts and salivary glands [18].

87 This finding suggests that *A. phagocytophilum* infection may regulate the levels of heme in a
88 tissue-specific manner with potential effects for pathogen and vector survival.

89

90 **Tick hemocyte metabolism in response to tick-borne pathogen infection**

91 Despite mammalian macrophages possess specialized effector functions to eliminate
92 invading microorganism, many intracellular bacteria infect and replicate inside this immune
93 cells [20]. Globally, macrophages can be divided into inflammatory (M1) and anti-
94 inflammatory (M2) macrophages [20]. Both macrophage types M1 and M2 can be infected
95 by intracellular bacteria; however, the metabolic profiles of these macrophages in response
96 to pathogenic stimulus are substantially different [20]. After stimulation with
97 lipopolysaccharide (LPS), macrophages M1 uptake more glucose via **glucose transporters**
98 (GLUTs, see Glossary) and increase the glycolytic flux and the production of lactate, a process
99 named **aerobic glycolysis**. In contrast, macrophages M2 have the metabolic profile
100 comparable to that of unstimulated cells, but with higher TCA-cycle and oxidative activity
101 [20].

102 Cellular immunity in ticks is mostly mediated by hemocytes, which are involved in the
103 phagocytosis of different microbes [21]. After establishing a primary infection in tick
104 midguts, *A. phagocytophilum* migrates to the salivary glands [11]. A secreted tick protein,
105 P11, enables *A. phagocytophilum* to infect tick hemocytes, which are required for the
106 migration of *A. phagocytophilum* from the gut to the salivary glands [22]. Isolation and *in*
107 *vitro* culture of tick hemocytes has remained elusive. Therefore, the study of tick hemocytes
108 metabolism in response to *A. phagocytophilum* infection has been based on the *I. scapularis*
109 ISE6 cell line. Based on the transcriptional response to *A. phagocytophilum*, ISE6 cells were
110 proposed to constitute a model of tick hemocytes involved in pathogen infection and
111 immune response [23,24]. Comparison of the metabolic profiles of macrophages M1 with
112 that of *A. phagocytophilum*-infected ISE6 cells suggests that the metabolic profile of tick
113 hemocytes in response to pathogen infection is very different to that of inflammatory
114 macrophages M1 (Fig. 1, Key Figure). Two major differences can be summarized: (i) ISE6 cells
115 infected by *A. phagocytophilum* do not display the aerobic glycolysis observed in mammalian

116 macrophages M1 and (ii) **hypoxia-inducible factor 1-alpha (HIF-1 α)** activation and
117 stabilization in ISE6 cells is not dependent of succinate accumulation and aerobic glycolysis
118 (Fig. 1). This suggests that the metabolic reprogramming associated with macrophage M1
119 activation does not occurs in tick hemocytes infected with *A. phagocytophilum*. Interestingly,
120 while 2-deoxyglucose (2DG), an inhibitor of **glycolysis**, inhibits HIF-1 α activation in
121 macrophages M1 [25], this compound has no effect on HIF-1 α activation in ISE6 cells [26].
122 Instead of aerobic glycolysis and succinate accumulation, HIF-1 α activity in *A.*
123 *phagocytophilum*-infected ISE6 cells appears to be mediated phosphoinositide 3-kinase
124 (PI3K) pathway activation (Fig. 1). In agreement with this, the PI3K inhibitor LY294002
125 decreased HIF-1 α DNA binding activity in infected ISE6 cells [26]. A metabolic property
126 shared by macrophages M1 and ISE6 cells is, however, an overall decrease in **tricarboxylic**
127 **acid cycle (TCA cycle)** activity upon bacterial infection [25,26].

128 The absence of immunogenic molecules such as LPS and diaminopimelic-type peptidoglycans
129 (DAP-PGN) in *A. phagocytophilum* and other bacteria transmitted by *I. scapularis* was
130 previously suggested to contribute to vector tolerance to tick-borne pathogens [7]. The lack
131 of these immune activators in tick-borne pathogens may help these microbes colonize ticks
132 without being eliminated [7]. Interestingly, lipids 1-palmitoyl-2-oleoyl-sn-glycero-3-
133 phosphoglycerol (POPG) and 1-palmitoyl-2-oleoyl diacylglycerol (PODAG) found in *A.*
134 *phagocytophilum* activate the immune deficiency (IMD) pathway in ticks [27]. Immune
135 priming with POPG and PODAG reduces the *A. phagocytophilum* burden in infected ISE6 cells
136 [27]. This shows that tolerance to infection can be associated with immunity and resistance
137 to tick-borne pathogens in ticks. This is expected considering that unchecked bacterial
138 multiplication within host cells could affect vector, and therefore, tick-borne pathogen
139 survival. Remarkably, recent research shows that IMD is involved in glucose and lipid
140 metabolism regulation in *Drosophila* [28] and constitutive activation of IMD induces a
141 significant reduction in the expression of genes involved in glycolysis, **gluconeogenesis** and
142 TCA cycle in flies [29]. Whether IMD activation by *A. phagocytophilum* regulates tick
143 hemocyte metabolism remains to be tested.

144

145 **Molecular mechanisms involved in tick metabolism modulation by tick-borne pathogens**

146 Tick metabolism modulation by tick-borne pathogens results mainly from the regulation of
147 the levels of metabolic enzymes. Although it remains a challenge to explain how pathogen
148 infection modify the level of thousands of tick genes and hundreds of tick proteins [30], the
149 molecular mechanisms behind this have been partially characterized using the model
150 bacteria *A. phagocytophilum*. To induce transcriptional reprogramming in infected cells, *A.*
151 *phagocytophilum* hijacks the transcriptional regulatory machinery of ticks by inducing the
152 activation of signaling pathways [31] and the recruitment of transcription factors [32],
153 metabolites [33], and histone modifying enzymes [34] (Fig. 2). *A. phagocytophilum* may also
154 induce the degradation of tick proteins by tick proteasome which in turn decreases the
155 levels of targeted metabolic enzymes [23]. In addition, *A. phagocytophilum* infection induces
156 a high proportion of alternatively spliced transcript events (ASEs), which increases the
157 predicted gene ontology processes related to lipids, lipoproteins, and cholesterol/sterol
158 metabolism and other processes in human HL-60 cells infected by this pathogen [35]. The
159 occurrence of ASEs has not been tested in *A. phagocytophilum*-infected ticks or tick cell lines
160 yet. However, there is no reason to rule out the hypothesis that ASEs also occur in ticks in
161 response to *A. phagocytophilum* infection.

162 Activation of tick signaling pathways by *A. phagocytophilum* involves G-protein-coupled-
163 receptor (GPCR) that stimulates *Ixodes* p21-activated kinase (IPAK1) through the activation
164 of PI3K which results in actin-mediated signaling [31]. Activation of IPAK1 results in actin
165 phosphorylation and accumulation of actin in the nucleus which mediates binding of RNA
166 polymerase II (RNAPII) and TATA box-binding protein (TBP) to the promoter of the gene
167 *salp16* inducing its expression [31,36]. PI3K activation was also suggested to induce the
168 expression of the genes *hif-1α*, *hif-1β* and acetyltransferase p300 upon *A. phagocytophilum*
169 infection in tick cells [26] (Fig. 2). Mobilization of HIF-1α, HIF-1β and p300 at HIF target genes
170 may induce HIF-mediated transcriptional activation of glycolytic genes [26].

171 Among the transcription factors (TFs) activated by *A. phagocytophilum* infection is the tick
172 transcriptional activator protein-1 (AP-1) which induces the expression of the genes *iafgp* (*I.*
173 *scapularis* antifreeze glycoprotein) and *kat* (kynurenine aminotransferase) in infected cells
174 [32]. KAT is an enzyme involved in the production of the tryptophan metabolite xanthurenic
175 acid [33]. Results suggests that xanthurenic acid acts as a co-factor of an uncharacterized TF

176 that regulates the expression of one organic anion transporting polypeptide (*oatp*) gene in *A.*
177 *phagocytophilum*-infected ISE6 cells [33]. The upregulation of *oatp* and *kat* was proposed to
178 be associated with increased levels of intracellular xanthurenic acid, which in turn favors *A.*
179 *phagocytophilum* replication in tick salivary glands [33]. Conversely, RNAi-mediated silencing
180 of *oatp* and *kat* expression decrease *A. phagocytophilum* levels in tick cells [33]. A general
181 mechanism is then proposed by which *A. phagocytophilum* nucleomodulins recruit tick
182 metabolites and TFs to activate target genes (Fig. 2).

183 The capacity of *A. phagocytophilum* to downregulate gene expression in mammalian
184 neutrophils was associated with host histone deacetylase 1 (HDAC1) recruitment to the
185 promoters of target genes by the bacterial nucleomodulin ankyrin repeat protein Anka [37].
186 Upon *A. phagocytophilum* infection, Anka is secreted through T4SS, enters the granulocyte
187 nucleus, binds stretches of AT-rich DNA and recruits host HDAC1 to its binding sites.
188 *A. phagocytophilum* infection also induces HDAC1 binding and deacetylation of histone H3
189 (H3) which in turn results in downregulation of many host defense genes including *CYBB*,
190 which encodes a component (i.e. NOX2) of the NADPH oxidase [37]. Downregulation of *CYBB*
191 reduces superoxide anion production by NADPH oxidase which is essential for
192 *A. phagocytophilum* survival in neutrophils [37]. Homologs of mammalian HDAC1 and H3
193 were identified in the *I. scapularis* genome [34]. Whether *A. phagocytophilum* Anka is
194 secreted and recruits HDAC1 to regulate gene transcription in infected ticks is currently
195 unknown. However, *Ixodes* HDAC1 is overrepresented in *A. phagocytophilum*-infected tick
196 salivary glands and chemical inhibition of this protein decreases *A. phagocytophilum* burden
197 in tick cells [34]. This shows that the expression and activity of HDAC1 is critical for *A.*
198 *phagocytophilum* survival in both mammalian and tick cells. Notably, in mammalian cells,
199 HDAC1 controls the expression of the metabolic enzyme fructose-1,6-bisphosphatase (FBP),
200 a rate-limiting enzyme in gluconeogenesis [38]. Interestingly, high HDAC1 protein levels [34]
201 were associated with low FBP protein levels in *A. phagocytophilum*-infected tick salivary
202 glands [26]. Conversely, low HDAC1 protein levels [34] correlated with high mRNA and
203 protein levels of *fbp* and FBP, respectively, in *A. phagocytophilum*-infected tick midguts [26].
204 The negative correlation between the protein levels of HDAC1 and that of *fbp* and FBP
205 strongly suggests that HDAC1 may be recruited by *A. phagocytophilum* nucleomodulins to
206 act as a repressor of *fbp* expression in tick salivary glands (Fig. 2). A decrease in FBP protein

207 levels may contribute to the inhibition of gluconeogenesis that *A. phagocytophilum* induces
208 in infected tick cells (see below) [23].

209

210 ***Tissue-specific changes of tick carbohydrate metabolism in response to infection***

211 Glucose is the most abundant carbohydrate in the hemolymph of adult ticks and it is
212 required in tick embryonic development [39]. The regulation of glucose metabolic pathways
213 in ticks is essential for *A. phagocytophilum* survival in the vector [23,26]. Notably, *A.*
214 *phagocytophilum* infection inhibits gluconeogenesis by decreasing the protein levels of
215 **phosphoenolpyruvate carboxykinase-cytosolic (PEPCK-C)** which results in lower levels of
216 glucose in *A. phagocytophilum*-infected ISE6 cells [23,26]. PEPCK-C is also a key regulatory
217 enzyme in **glyceroneogenesis** [40] and thus, low levels of PEPCK-C were associated with
218 reduced glycerol 3-phosphate production in infected tick cells [41]. Low PEPCK-C activity and
219 decreased glucose anabolism inhibits apoptosis which is essential for *A. phagocytophilum*
220 survival in tick cells and tissues [23,30]. Interestingly, glycerol 3-phosphate levels were also
221 reduced in tick nymphs infected by *Borrelia mayonii* (Table 1). Low levels of glycerol 3-
222 phosphate may have a significant impact in fatty acids metabolism, a poorly explored facet
223 in tick-pathogen interactions.

224 Tissue-specific differential regulation of apoptosis pathways was observed in adult female
225 midguts and salivary glands in response to *A. phagocytophilum* infection [42]. Likewise, the
226 transcriptional reprogramming induced by *A. phagocytophilum* affects the levels of
227 carbohydrate metabolism genes and proteins in a tissue-specific manner [26]. For example,
228 the protein levels of all glycolytic enzymes increase in *A. phagocytophilum*-infected tick
229 midguts, except for the cofactor-independent phosphoglycerate mutase (iPGM) and
230 pyruvate kinase (PK) that despite gene up-regulation, the proteins were under-represented
231 in this tissue [26]. In addition, gene and protein levels of phosphofructokinase (PFK) did not
232 change in tick midguts in response to infection [26]. However, in salivary glands, the
233 glycolysis enzymes fructose-bisphosphate aldolase A (ALDA), PFK and hexokinase (HXK) were
234 under-represented while the rest of the glycolytic enzymes were over-represented. To
235 accurately assess the metabolic impact of the differential protein representation in tick

236 midguts and salivary glands, a tissue-specific metabolomics approach is required. However,
237 the lower levels of PK and HXK, key regulatory enzymes in glycolysis, in infected tick midguts
238 and salivary glands, respectively, suggest that the production of glycolytic intermediates may
239 be affected in a tissue-specific manner. It would be also important to assess the enzymatic
240 activity of key enzymes which may vary in *A. phagocytophilum*-infected cells and tissues. For
241 example, it was reported that the activity of HXK decreases more than two-folds in *A.*
242 *phagocytophilum*-infected ISE6 cells [43]. The transcriptional activation of tick glycolytic
243 genes was proposed to be mediated by tick HIF-1 α [26], which contains structural domains
244 conserved in model organisms and the gene *hif-1 α* was significantly upregulated in infected
245 tick midguts [26]. Another important gene targeted by HIF-1 α is pyruvate dehydrogenase
246 kinase 1 (*pdk1*) which encodes the protein PDK1 that controls the enzymatic activity of
247 pyruvate dehydrogenase E1 (PDE1) which transforms pyruvate into **Acetyl-CoA**, the first step
248 in the TCA cycle. PDK1 mRNA and protein levels were higher in *A. phagocytophilum*-infected
249 tick salivary glands suggesting that this pathogen inhibits the TCA cycle in infected salivary
250 glands [26].

251

252 ***Crosstalk between tick amino acid and carbohydrate metabolism during pathogen*** 253 ***infection***

254 Several recent studies have shown that amino acids are central to host-pathogen metabolic
255 interaction [3,23,41,44]. When digested, the protein-rich diet of ticks generates large
256 amounts of amino acids. Therefore, amino acid metabolism is of great interest in ticks. For
257 example, tyrosine-associated toxicity is lethal for blood-sucking arthropods which points out
258 the significance of tyrosine degradation pathways. Inhibition of tyrosine aminotransferase
259 (TAT) and 4-hydroxyphenylpyruvate dioxygenase (HPPD), the first two enzymes of the
260 phenylalanine/tyrosine degradation pathway, caused the death of mosquitoes, kissing bugs
261 and ticks after a blood meal [45].

262 Infection by *A. phagocytophilum* rewires the network of tick vector cell processes and
263 changes the relative importance of some biological pathways including those involved in
264 metabolism [46]. This is expected considering that *A. phagocytophilum* has a small genome

265 and cannot actively carry out several metabolic processes, and therefore requires and
266 hijacks tick cell resources for survival within the vector [47]. For example, out of twenty
267 amino acids, the *A. phagocytophilum* genome encodes only for the enzymes responsible of
268 the biosynthesis of four amino acids (proline, glutamine, glycine and aspartate) and the
269 glycolysis enzymes are reduced to those that produce glyceraldehyde-3-phosphate and
270 dihydroxyacetone phosphate (DHAP) from **phosphoenolpyruvate** (PEP) [47]. In
271 consequence, *A. phagocytophilum* changes the levels of amino acid metabolism enzymes
272 [41], which in turn affects the intracellular levels of several amino acids and metabolic
273 intermediates [23,33]. Interestingly, tick cells use tyrosine as a fuel to synthesize PEP during
274 early *A. phagocytophilum* infection [41]. This process is achieved by activating the
275 tyrosine/**oxaloacetate** (OAA)/PEPCK-M/PEP node by which *A. phagocytophilum* infection
276 may decrease the tyrosine pool, which in turn may protect ticks against tyrosine-induced
277 toxicity *in vivo* [41].

278 Tick-borne flaviviruses are known to affect protein processing and amino acid metabolism in
279 tick cells [48,49]. Particularly, infection by Langat virus (LGTV) increased protein levels of
280 acetyl-CoA acetyltransferase 1 (ACAT1), aldehyde dehydrogenase (DP5CD), glutamate
281 dehydrogenase (GLUD1) and fumarylacetoacetase (FAH) in ISE6 cells [49]. Knockdown of the
282 gene *acat1* was associated with decreased LGTV genome replication and LGTV particle
283 release, while knockdown of the gene *fah* decreased only LGTV particle release in ISE6 cells
284 [48]. Interestingly, FAH catalyzes the last step in tyrosine degradation suggesting that
285 flaviviruses infection may, similarly to *A. phagocytophilum*, contribute to tyrosine
286 detoxification in ticks.

287

288 ***Tick lipid metabolism is exploited by different tick-associated microorganisms***

289 Starvation and desiccating conditions that ticks have to endure during off-host periods affect
290 the energy reserves of the tick [50]. For example, starvation of *Dermacentor variabilis* ticks in
291 optimal humidity conditions is known to result in up to 60% losses in lipid reserves [50]. The
292 parietal fat body, the main lipid reserve organ for triacylglycerol and cholesterol, is gaining

293 increasing interest for the study of infections in insects and ticks [51,52]. However, lipid
294 metabolism signaling pathways are still rarely studied in ticks.

295 Fatty acids play important roles as energy sources for several microorganisms including
296 pathogenic bacteria [53]. If a pathogen can prevent the use of energy reserves by the host,
297 this will affect its immune system, therefore promoting pathogen infection and persistence.
298 *B. burgdorferi* is not able to synthesize fatty acids and cholesterol, thus relying on the
299 vertebrate host cell or the blood meal of the tick for lipids supply [54]. Similarly, host
300 cholesterol and other lipids are required by *A. phagocytophilum* for infection and
301 multiplication in human cells [55]. Perilipin (PLIN), a major adipocyte lipid droplet-associated
302 phosphoprotein that plays a central role during lipolysis and cholesterol synthesis, is
303 differentially expressed in HL-60 cells in response to infection with *A. phagocytophilum* [56].
304 The bacteria are able to alter PLIN levels in infected cells, thus modifying the lipid
305 metabolism of the host to facilitate bacterial infection [56]. This phosphoprotein is likely to
306 play a similar role in the metabolism of the tick although its role has not been characterized
307 so far.

308 Other obligate intracellular pathogens such as viruses need to hijack the cellular machinery
309 for the synthesis of lipids to complete their replication. Flavivirus life cycle is very closely
310 associated to host cell lipids, and they can rearrange intracellular membranes and reorganize
311 the host cell lipid metabolism of infected cells to ensure viral replication [57]. Dengue virus
312 infection can alter metabolic pathways in humans such as fatty acid biosynthesis and **β -**
313 **oxidation** [58]. Exosomes are small extracellular vesicles that appear to play a central role in
314 cell-to-cell communication [58]. Exosomes transport bioactive molecules, such as messenger
315 RNA (mRNAs) and microRNA (miRNAs) that can be transferred in active form to adjacent
316 cells or to distant organs thus being important vehicles for metabolic cross-talk [59]. Recent
317 research has shown that Flaviviruses use arthropod-derived exosomes secreted by tick cells
318 as a mean for viral RNA and metabolite transmission from the vector, and the vertebrate
319 exosomes for dissemination within the host [60]. The knockdown of transcripts for a
320 glycosylphosphatidylinositol (GPI)-anchored protein (VNN) was associated with decreased
321 LGTV genome replication and LGTV release in ISE6 tick cells [48]. VNN is involved in
322 coenzyme-A (CoA) metabolism [61], which is critical for lipid metabolism.

323 Fungal infections can also modulate the lipid metabolism of the host [62]. Recent research
324 with different isolates of *Metarhizium anisopliae* showed that infection of *Rhipicephalus*
325 *microplus* ticks with entomopathogenic fungi can modulate the lipid content thus inducing
326 changes in important metabolic pathways [62]. However, lipid metabolism modulation
327 during *M. anisopliae* infection in ticks was independent of AMP-activated protein kinase
328 (AMPK) and extracellular signal-regulated protein kinase (ERK) pathways [62] which are the
329 major pathways involved in the breakdown of triacylglycerol into fatty acids in mammals [63,
330 64]. This suggested that tick lipid metabolism modulation by *M. anisopliae* occurs through
331 alternative and uncharacterized lipolysis pathway.

332

333 **Concluding Remarks**

334 Interactions between ticks, hosts and pathogens involve many metabolic pathways such as
335 carbohydrate, protein, lipid and redox that are affected to produce conflict and cooperation
336 between them. These interactions at the metabolic level lead to mutual beneficial effects of
337 the tick-hosts-pathogen molecular interactions. Among them, amino acids are central to the
338 host-pathogen metabolic interaction. Nevertheless, synthesis and degradation of amino
339 acids is narrowly connected to carbohydrate metabolism, and the later with the lipid
340 metabolism.

341 Despite recent advances in the study of tick-host-pathogen molecular interactions, most of
342 the pathways and molecules involved in these interactions are not fully characterized.
343 Furthermore, the regulatory mechanisms affecting the metabolic pathways affected by
344 these interactions are only partially characterized (see Outstanding Questions). Future
345 directions should include the application of omics technologies together with data
346 integration algorithms [46] to the investigation of tick interactome, regulome, miRNAome,
347 epigenome and metabolome and their interactions to advance the metabolic perspective on
348 tick-pathogen interactions.

349 The identification of the metabolic adaptations during tick-pathogen interactions that
350 support tick survival, spread and pathogen infection and transmission could provide new
351 therapeutic targets for the prevention and control of tick-borne diseases. Studies of the

352 interactions between the pathogen and the host lipid metabolism can help to identify
353 potential targets for the development of vaccines or treatments against health threatening
354 pathogens. These strategies could include the inhibition of lipid biosynthesis. However,
355 manipulation of this major metabolic pathway can also be detrimental for the host. For
356 instance, Lovastatin treatment, an inhibitor of cholesterol synthesis, appears to delay
357 infection and increases survival rates in AG129 mice infected with Dengue Virus Serotype 2
358 while it also tends to increase viremia which could affect disease progression [65]. A better
359 understanding of the interactions of pathogens with the host cell lipid metabolism and other
360 metabolic pathways could provide new therapeutic targets to control infections by tick-
361 borne pathogens. Furthermore, as recently proposed the application of machine learning
362 and big data analysis to the tick-host-pathogen interactions datasets may also lead to the
363 high throughput identification of candidate vaccine protective antigens [66].

364

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367

368

369 **Glossary**

370 **Tricarboxylic acid cycle (TCA cycle):** a central route for oxidative phosphorylation in cells. In
371 the TCA cycle, acetyl-CoA is oxidized into carbon dioxide and reducing factors (NADH and
372 FADH₂), that contribute to synthesis of ATP during oxidative phosphorylation, are produced.
373 The TCA cycle is a crossroad of cellular metabolism in which carbohydrate, fatty acids and
374 amino acid metabolisms are interconnected.

375 **β-Oxidation:** the major pathway for the degradation of fatty acids and is essential to
376 produce energy from the generation of acetyl-CoA, NADH and FADH₂ when glucose supply is
377 limiting.

378 **Gluconeogenesis:** a metabolic pathway that during a longer-term fast or starvation results in
379 *de novo* glucose synthesis from non-carbohydrate carbon substrates, mainly lactate and
380 glycerol.

381 **Glyceroneogenesis:** a metabolic pathway that is activated during glucose deprivation to
382 synthesize glycerol 3-phosphate, a building block of triglyceride and fatty acid. In this
383 pathway, glycerol 3-phosphate is synthesized from pyruvate, alanine, glutamine and TCA
384 cycle intermediates.

385 **Glycolysis:** a major catabolic pathway in which one molecule of glucose is transformed into
386 two molecules of pyruvate releasing in the process two net molecules of ATP and two of
387 NADH. The expression of glycolytic genes is regulated by HIF-1α.

388 **Aerobic glycolysis:** A term frequently used to name a metabolic profile similar to that of the
389 *Warburg effect* in which a cell, even under normoxic conditions, generates high amount of
390 energy by using the glycolytic pathway, notably with high production of lactate.

391 **Phosphoenolpyruvate carboxykinase (PEPCK):** PEPCK catalyzes the first committed step in
392 gluconeogenesis, in which OAA is converted into PEP. There are two isoforms of the enzyme:
393 mitochondrial (PEPCK-M) and cytosolic (PEPCK-C). PEPCK-C is a key regulatory enzyme of
394 glyceroneogenesis and gluconeogenesis.

395 **Hypoxia-inducible factor 1-alpha (HIF-1 α):** a subunit of the heterodimeric transcription
396 factor HIF-1 that together with HIF-1 β regulates the cellular response to hypoxia by inducing
397 the transcriptional activation of genes involve in glycolysis.

398 **Acetyl-CoA:** a central metabolic intermediate synthesized in the mitochondria. Acetyl-CoA is
399 the molecule through which glycolytic pyruvate enters the TCA cycle to be oxidized for
400 energy production. It can be derived from carbohydrates, fatty acids and amino acids.

401 **Glucose transporters (GLUT):** a wide group of membrane proteins that transport glucose
402 across the cell membrane. The GLUT family is divided into three classes of which class I
403 member GLUT1 is transcriptionally regulated by HIF-1 α .

404 **Oxaloacetate (OAA):** a central metabolite produced in the TCA cycle that takes part in
405 gluconeogenesis, amino acid metabolism and fatty acid synthesis among other pathways. In
406 addition, OAA and PEP result from anaplerotic reactions that keep the metabolic flux of
407 glycolysis and gluconeogenesis.

408 **Phosphoenolpyruvate (PEP):** a key metabolite that stands at the crossroad between
409 gluconeogenesis, glycolysis and TCA cycle and can be produced by the activity of two
410 enzymes enolase and PEPCK.

411

412

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583

584 **Figure legends**

585 **Figure 1. Metabolic profiles of tick hemocytes and mammalian pro-inflammatory**
586 **macrophages M1.** The figure displays the metabolic profile of *A. phagocytophilum* (bacteria
587 without LPS)-infected ISE6 cells, a model of tick hemocytes, compared to that of mammalian
588 macrophages M1 in response to other intracellular bacteria with LPS. High levels of
589 hexokinase (HXK, green) and phosphofruktokinase (PFK, green) are associated with high
590 aerobic glycolysis and increased lactate production in mammalian macrophages M1 [25].
591 The levels and activity of tick HXK decreased (red), and the levels of tick PFK did not change
592 (black), in response to infection which was associated with lower levels of lactate in infected
593 ISE6 cells [23,26,43]. HIF-1 α activation and stabilization (green), that enhances the
594 expression of glycolytic genes, is mediated by aerobic glycolysis and succinate accumulation
595 from glutamine in macrophages M1 [25]. In consequence, blocking aerobic glycolysis with 2-
596 deoxyglucose (2DG) inhibits HIF-1 α activation in these macrophages [25]. In contrast, 2DG
597 does not inhibit HIF-1 α activation (green) in ISE6 cells, an effect that is achieved by

598 LY294002, an inhibitor of the phosphoinositide 3-kinase (PI3K) pathway [26]. This suggests
599 that HIF-1 α activity in tick hemocytes infected with *A. phagocytophilum* is regulated by PI3K
600 and not aerobic glycolysis. Macrophages M1 and ISE6 cells share a low TCA cycle activity [25,
601 26]. Vertical arrows represent decrease (red) and increase (green) in metabolite levels.

602

603 **Figure 2. Molecular mechanisms potentially implicated in tick metabolism modulation by**
604 **the model pathogen *A. phagocytophilum*.** Molecular mechanisms proposed to participate in
605 the transcriptional reprogramming and proteome modulation induced by *A. phagocytophilum*
606 infection in tick cells. Firstly, *A. phagocytophilum* activates the phosphoinositide 3-kinase
607 (PI3K) signaling pathway in a G-protein-coupled-receptor (GPCR)-dependent manner to
608 induce the expression of *salp16* [31], and hypoxia-inducible factor (HIF) *hif-1 α* in ticks [26]
609 (sequential steps and tick molecular factors colored in violet). Secondly, *A. phagocytophilum*
610 recruits tick transcription factors (TF), such as transcriptional activator protein-1 (AP-1) [32]
611 and metabolites, such as xanthurenic acid [33], to regulate the expression of the metabolic
612 genes *oatp* [33] and *kat* [32] (sequential steps and tick molecular factors colored in blue).
613 Other tick metabolic genes may be regulated by the same mechanism in which we propose
614 that *A. phagocytophilum* uncharacterized nucleomodulins may participate in the recruitment
615 of tick TFs. Thirdly, the recruitment of histone deacetylase 1 (HDAC1) to the promoters of
616 target genes through the activity of nucleomodulins may contribute to the repression of tick
617 genes, e.g. fructose-1,6-bisphosphatase (*fbp*) (sequential steps and tick molecular factors
618 colored in green). Lastly, although the mechanism is unknown, *A. phagocytophilum* also
619 targets tick proteins to the proteasome which in turn may result in the underrepresentation
620 of targeted metabolic enzymes [23]. Abbreviations are as follow: *Ixodes* p21-activated kinase
621 (IPAK1), RNA polymerase II (RNAPII), TATA box-binding protein (TBP), acetyltransferase p300
622 (p300). Closed and open chromatin were represented as red and green double-stranded
623 DNA, respectively.

624

625

Table 1. Tick metabolic pathways affected by tick-borne pathogens

Metabolic pathways	Experimental system (<i>in vitro</i> vs. <i>in vivo</i>)	Affected by (name of pathogen species)	Effect on tick metabolism*	References
Glycolysis	ISE6 tick cells	<i>Anaplasma phagocytophilum</i>	Decreased levels and activity of HKX	[26,43]
			Increased levels of PEP	[41]
			Decreased levels of glucose and lactate	[23]
	<i>Ixodes scapularis</i> adults midguts		Upregulation of genes involved in glycolysis (except <i>hvk</i> and <i>pfk</i>)	[26]
			Upregulation of genes <i>hif-1α</i> and <i>hif-1β</i>	
			Increased protein levels of glycolytic enzymes (except iPGM, PK and PFK) and glucose transporters GLUT1A and SGLT2	
<i>Ixodes scapularis</i> adults salivary glands	Upregulation of genes involved in glycolysis (except <i>hvk</i> , <i>pfk</i> , <i>alda</i> , <i>pgk1</i> and <i>enol</i>)	[26]		
	Increased protein levels of glycolytic enzymes (except HKX, PFK and ALDA) and glucose transporter GLUT1A			
Gluconeogenesis	ISE6 tick cells	<i>Anaplasma phagocytophilum</i>	Decreased levels of PEPCK-C and glucose	[23]
			Increased protein levels of PEPCK-M	[26]
	<i>Ixodes scapularis</i> adults midguts		Upregulation of genes involved in gluconeogenesis	[26]
Decreased levels of gluconeogenesis enzymes (except FBP)				

	<i>Ixodes scapularis</i> adults salivary glands		Decreased levels of gluconeogenesis enzymes	
Glyceroneogenesis	ISE6 tick cells	<i>Anaplasma phagocytophilum</i>	Decreased levels of Glycerol 3-phosphate	[26]
	<i>Ixodes scapularis</i> adults midguts		Increased levels of PEP which may inhibit the enzyme TPI	
	<i>Ixodes scapularis</i> adults salivary glands		Upregulation of genes <i>tpi</i> and <i>gpdh</i> Increased and decreased protein levels of TPI and GPDH, respectively	
	<i>Ixodes scapularis</i> nymphs**	<i>Borrelia mayonii</i>	Upregulation of gene <i>tpi</i> Increased protein levels of TPI and GPDH	[54]
Lipids	<i>Rhipicephalus microplus</i> adults fat body	<i>Metarhizium anisopliae</i>	Increased levels of cholesterol ester and triacylglycerol Decreased levels of free cholesterol	[51]
	<i>Ixodes scapularis</i> nymphs**	<i>Borrelia mayonii</i>	Decreased levels of cholesterol, ethanolamine phosphate and ethanolamine	[54]

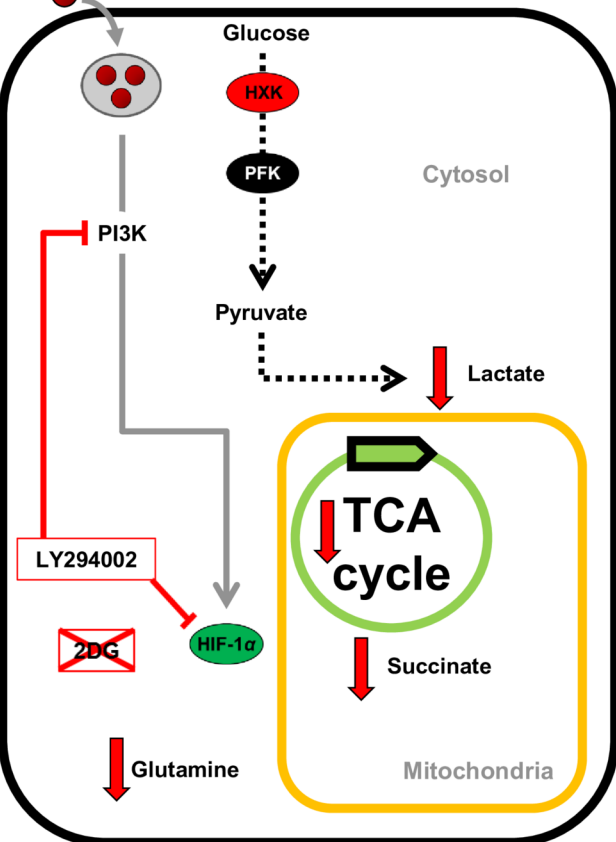
Amino Acids	ISE6 tick cells	Anaplasma phagocytophilum	Decreased levels of alanine, glycine, glutamine, glutamate and methionine Increased levels of serine, proline, tyrosine and phenylalanine Tyrosine is used as fuel to synthesize PEP	[23,41]
			Upregulation of gene <i>oatp</i> Xanthurenic acid acts as a co-factor for unknown transcription factor(s) that control the expression of <i>oatp</i> KAT regulates the expression of <i>oatp</i>	[33]
	<i>Ixodes scapularis</i> adults midguts and salivary glands		Changes in the levels of genes and proteins involved in amino acid synthesis and degradation	[41]
	<i>Ixodes scapularis</i> nymphs salivary glands		Upregulation of genes <i>oatp</i> and <i>kat</i> Xanthurenic acid acts as a co-factor for unknown transcription factor(s) that control the expression of <i>oatp</i>	[33]
	<i>Ixodes scapularis</i> nymphs**	Borrelia mayonii	Decreased levels of alanine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, glutamate, glutamine and glycine Increased levels of tryptophan and glutamine	[54]
		Borrelia burgdorferi	Decreased levels of alanine, isoleucine, leucine, phenylalanine, proline, threonine, valine and glycine	

Redox	BME26 tick cells	<i>Anaplasma marginale</i>	Downregulation of pro-oxidant genes and upregulation of antioxidant genes	[67]
	<i>Ixodes scapularis</i> adults midguts and salivary glands	<i>Anaplasma phagocytophilum</i>	Significant changes in the levels of genes and proteins of hemoglobinolytic enzymes affecting host hemoglobin levels in tick tissues	[18]

* Full name of enzymes are in the main text except for phosphoglycerate kinase 1 (PGK1), enolase (ENOL), glycerol-3-phosphate dehydrogenase (GPDH), triosephosphate isomerase (TPI). ** Only the levels of tick metabolites in response to *Borrelia* spp. infection at day 4 of feeding were considered [54].

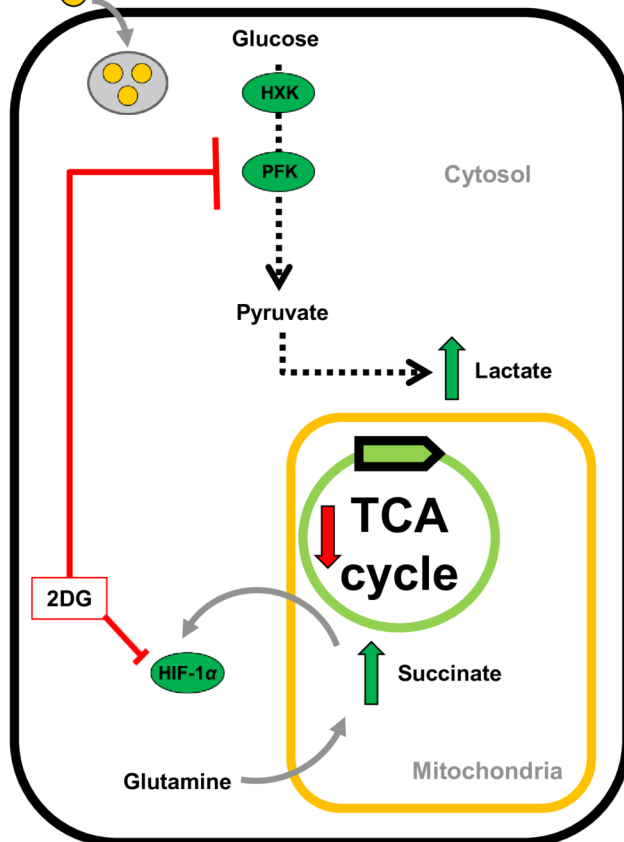
ISE6 cells - tick hemocytes model

A. phagocytophilum no LPS



Mammalian macrophages M1

Bacteria with LPS



A. phagocytophilum

Tick cell

GPCR

PI3k

IPAK1

Actin

Actin^P

TF

AnkA

Cytosol

Nucleus

TF

RNAPII

Activation
(*oatp* and *kat*)

HDAC1

Repression
(*fbp*)

Actin^P

TBP

RNAPII

Activation
(*salp16* and *hif-1 α*)

p300

HIF-1 β

HIF-1 α

RNAPII

Activation
(glycolytic genes)

Molecular factors



Bacterial nucleomodulins



Xanthurenic acid



Phosphate



Tick proteins

Molecular processes



Phosphorylation



Recruitment of tick proteins
and/or metabolites



Targeting tick proteins
to proteasome degradation

