PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM

***Interpretive Summary*: Mechanisms linking metabolic stress with innate immunity in the endometrium, Sheldon et al.**

Rapid and robust immune responses are required to resist bacterial infections of the uterus after parturition in dairy cattle. However, metabolic stress increases the risk of developing metritis and endometritis. Here we review the evidence for cellular mechanisms that link metabolic stress with innate immunity in the endometrium. We suggest that stressing cellular metabolism increases the risk of disease by impairing innate immunity.

PHYSIOLOGY AND ENDOCRINOLOGY SYMPOSIUM: Mediators of Effects of Stress on Reproduction, Growth, and Lactation

Invited Review: Mechanisms linking metabolic stress with innate immunity in the endometrium

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ABSTRACT

Bacterial infections of the uterus after parturition are ubiquitous in dairy cattle and often cause uterine disease, such as metritis or endometritis. However, the metabolic stress associated with milk production increases the risk of developing disease. Resolution of bacterial infections requires rapid and robust innate immune responses, which depend on host cell receptors recognizing pathogen-associated molecular patterns, such as lipopolysaccharide (LPS) from Gram-negative bacteria. Here, we argue that metabolic stress impairs the inflammatory response to pathogens. Glucose and glutamine are the major energy sources for cells, but their abundance is reduced in postpartum dairy cows. Furthermore, inflammatory responses exacerbate metabolic stress, with animals and tissues consuming more glucose when challenged with LPS. However, depriving endometrial tissue of glucose or glutamine impairs the secretion of interleukin (IL)-1β, IL-6 and IL-8 in response to pathogen-associated molecular patterns. Glycolysis and the intracellular sensor of energy, AMPK, are important for the response to LPS because perturbing glycolysis or AMPK activity reduces the secretion of IL-1β, IL-6 and IL-8 in the endometrium. The mevalonate pathway for cellular cholesterol synthesis may also be linked to immunity as inhibition of the terminal enzyme in the pathway, squalene synthase, reduces inflammatory responses to pathogenic bacteria and LPS. In contrast, only modest effects on inflammation are found when modulating the sensor of cellular nutrient satiety, mammalian target of rapamycin (mTOR), or the endocrine regulator of metabolism, insulin-like growth factor-1. We suggest that stressing cellular metabolism increases the risk of uterine disease by impairing endometrial defenses.

Key Words: Uterus, bacteria, inflammation, metabolism

INTRODUCTION

Bacterial infections of the uterus are ubiquitous after parturition in dairy cattle, and they often cause metritis and endometritis (Sheldon et al., 2009). Treating metritis, the subsequent infertility, and reduced milk production, costs the EU and USA dairy industries about $2 billion/annum (Sheldon et al., 2009). The risk of developing uterine disease is increased by the metabolic stress associated with milk production (LeBlanc, 2012; Wathes, 2012; Esposito et al., 2014). Countering bacterial infections requires rapid and robust inflammatory responses, driven by the innate immune system (Sheldon et al., 2017). The systems of energy metabolism, innate immunity, and reproduction are highly integrated because they have co-evolved over millennia, and so stress in one system, may affect the others. Here, we argue that cellular metabolic stress impairs the inflammatory response to pathogens in the endometrium.

There are many informative epidemiological and whole-animal studies on the associations between dairy cow nutrition and disease, particularly in the transition period and early lactation. Reviewing the in vivo studies is beyond the scope of the present paper, and we refer readers to comprehensive reviews that encapsulate this information (Chagas et al., 2007; Sordillo et al., 2009; LeBlanc, 2012; Esposito et al., 2014). Here, we focus on tissue and cells, and review the mechanisms that may link cellular metabolic stress with innate immunity in the endometrium. Evidence is presented for the roles of glucose and glutamine in immunity, and how intracellular sensors of metabolism, hormones, and cholesterol may modulate cellular defense. We suggest that stressing cellular metabolism increases the risk of uterine disease by impairing endometrial defenses.

METABOLIC STRESS

The modern dairy cow is under metabolic stress because the energy demand of lactation is typically three times the resting metabolic rate. For a typical dairy cow producing 40 liters milk/day, the metabolic energy requirements for milk production are about 200 MJ/day, whereas only about 65 MJ/day is needed for maintenance. An equivalent metabolic demand for humans is running three marathons every day, because the energy expenditure to complete a marathon is almost the same as the runner's daily energy intake (Williams et al., 1984). Although dairy cattle have been genetically selected to produce milk, they simply cannot consume enough food to meet the metabolic demand of lactation. Consequently, body tissues are broken down to supply metabolic energy and satisfy the "negative energy balance" (LeBlanc, 2012; Wathes, 2012; Esposito et al., 2014). Negative energy balance is reflected by reduced blood concentrations of glucose, glutamine, and insulin-like growth factor 1 (**IGF-1**) in postpartum animals (Doepel et al., 2006; Chagas et al., 2007; Wathes et al., 2011; Ingvartsen and Moyes, 2013). Furthermore, metabolic stress during early lactation in dairy cattle is associated with an increased incidence of uterine disease (Chagas et al., 2007; Kerestes et al., 2009; Galvao et al., 2010). Even more striking is that reduced appetite before calving increases the risk of severe uterine disease after parturition (Huzzey et al., 2007).

UTERINE DISEASE AND INNATE IMMUNITY

All cows have abundant bacteria in the uterine lumen after parturition (Sheldon et al., 2002). These bacteria come not only from the environment but may also be part of a resident uterine microflora (Karstrup et al., 2017; Moore et al., 2017). However, high-milk-yield cows have a propensity for developing bacterial disease of the endometrium. Up to 40% of animals develop metritis within 10 days of parturition, chronic endometritis persists >3 weeks post

partum in about 20% of cows, and subclinical endometritis affects at least an additional 15% of animals (LeBlanc et al., 2002; Sheldon et al., 2002; Kasimanickam et al., 2004; Zwald et al., 2004; Sheldon et al., 2009). Based on culture and molecular techniques, key bacteria in the pathogenesis of postpartum uterine disease include endometrial pathogenic *Escherichia coli*, *Trueperella pyogenes* and anaerobic bacteria, such as *Fusobacteria*, *Prevotella* and *Bacteroides* species (Noakes et al., 1991; Sheldon et al., 2002; Sheldon et al., 2010; Amos et al., 2014; Bicalho et al., 2017).

Bacteria are sensed by the innate immune system, with their pathogen-associated molecular patterns detected by pattern recognition receptors, such as Toll-like receptors (**TLR**s) on endometrial cells, neutrophils and macrophages (reviewed in: Sheldon et al., 2017). For example, TLR4 binds the lipopolysaccharide (**LPS**) endotoxin of *E. coli*, and TLR2, TLR1 and TLR6 bind bacterial lipopeptides. Activation of TLR signaling in endometrial cells leads to the secretion of prostaglandins and interleukin (**IL**)-1β, IL-6 and IL-8 (Herath et al., 2006; Herath et al., 2009; Cronin et al., 2012; Turner et al., 2014). However, the response to pathogen molecules is energetically expensive *in vivo* and *in vitro* (Turner et al., 2016; Kvidera et al., 2017). A striking example is that animals require an additional kilogram of glucose to supply the activated immune system in the first 12 h after in vivo challenge with LPS (Kvidera et al., 2017). Unfortunately, uterine disease further exacerbates metabolic stress because cows with metritis eat 2 Kg/day less food (Wittrock et al., 2011). There are also metabolic interactions between host and pathogens because tissues and bacteria compete for the same nutrients, and pathogens can sense and adapt to metabolic changes in tissues (Olive and Sassetti, 2016).

Rapid and robust inflammatory responses are important to resist bacterial infections effectively and return animals to health (Medzhitov, 2008; Chovatiya and Medzhitov, 2014; Sheldon et al., 2017). Delayed or inadequate acute inflammatory responses can lead to problems, including failure to clear microbes, disrupting the active resolution of inflammation, and delaying the repair of inflamed and damaged tissues. Missing the opportunity to counter infections effectively often leads to chronic inflammation (Fig. 1). Indeed, chronic endometritis is a common sequel to postpartum uterine infections in dairy cows (Chagas et al., 2007; Kerestes et al., 2009; Sheldon et al., 2009; LeBlanc, 2012). Furthermore, animals manipulated to have severe negative energy balance had increased abundance of inflammatory genes in the endometrium 2 weeks post partum, whereas animals in mild negative energy balance appeared to have recovered by that time (Wathes et al., 2009; Wathes, 2012).

Innate immunity is optimally tuned when metabolism is in homeostatic balance (Chovatiya and Medzhitov, 2014; O'Neill et al., 2016). So, compromised function of peripheral blood neutrophils may be one explanation for how negative energy balance is linked to uterine disease (Hammon et al., 2006; Galvao et al., 2010; LeBlanc, 2012; Mendonca et al., 2013). However, nutrient availability often has subtle effects on circulating immune cells and there is considerable variation amongst studies. Furthermore, we reason that the persistence of neutrophils may reflect a failure in the innate immune system to return the postpartum endometrium to homeostasis. Our alternative concept is that stressing cellular metabolism increases the risk of disease by impairing defenses in the tissue and cells of the endometrium.

GLUCOSE AND GLUTAMINE

Glucose and glutamine are the major carbon substrates used by most cells for metabolic energy (Wellen et al., 2010; O'Neill et al., 2016). Glycolysis and the Krebs cycle yield NADH and ATP from glucose (Fig. 2). Glutamine is anapleurotic; supplying substrate for the Krebs cycle following glutaminolysis to glutamate, and conversion of glutamate to α-

ketoglutarate (DeBerardinis and Cheng, 2010; Zhang et al., 2017). During innate immune responses to LPS, macrophages and monocytes further increase the supply of ATP and anabolic substrates by accelerating glucose flux to lactate via aerobic glycolysis, which is often called the Warburg effect (Tannahill et al., 2013; Turner et al., 2016). In the endometrium, the response to LPS is similarly energetically expensive, with endometrial tissue and cell cultures consuming additional glucose and inducing the Warburg effect (Turner et al., 2016). However, in postpartum dairy cows under metabolic stress the abundance of glucose and glutamine is often reduced in the peripheral circulation (Doepel et al., 2006; Ingvartsen and Moyes, 2013). Furthermore, it is the local abundance of glucose and glutamine in the endometrial tissue that is important, and this may be further reduced in damaged tissues after parturition.

Exciting evidence is emerging for integration of metabolism with innate immunity in immune cells, including dendritic cells and macrophages (Krawczyk et al., 2010; Tannahill et al., 2013; O'Neill et al., 2016). Our recent studies using bovine endometrium also examined the impact of glucose and glutamine abundance on innate immunity (Fig. 3) (Turner et al., 2016; Noleto et al., 2017). Limiting the availability of glucose to ex vivo organ cultures of endometrium reduces the secretion of IL-1β, IL-6 and IL-8 in response to LPS or lipopeptides (Turner et al., 2016). Similarly, depletion of glutamine, in the presence of abundant glucose, reduced the IL-1β, IL-6 and IL-8 response to LPS by at least 50% (Noleto et al., 2017). A key finding in these studies was that the abundance of glucose and glutamine were more important for inflammatory responses than their absolute concentrations. The availability of glucose and glutamine to cells depends on several transmembrane glucose and glutamine transporters, which are regulated by intracellular regulators of metabolism (Zoncu et al., 2011; Hardie et al., 2012). Nutrient transporters are also regulated by several hormones such as insulin and IGF-1; although, dairy cows are often insulin-resistant after partition (Kerestes et al., 2009; De Koster and Opsomer, 2013; Forde et al., 2014). Exploring the impact of the abundance of nutrients, rather than nutrient concentrations, is a conceptual change because most dairy cow studies focus on monitoring or mimicking blood concentrations of nutrients. However, we propose that immune responses depend on the abundance of nutrients entering into the cells of the postpartum endometrium, and their flux through the cellular metabolic pathways.

As might be expected, in the absence of both glucose and glutamine endometrial inflammatory responses to LPS are markedly reduced (Noleto et al., 2017). However, supplying glucose alone increased inflammation, whereas supplying glutamine alone did not. The dependence of glutamine metabolism on glycolysis was confirmed using 2-deoxy-D-glucose to inhibit glycolysis, which prevented inflammatory responses to LPS in endometrial organ cultures (Noleto et al., 2017). Similar observations of the importance of glycolysis to facilitate glutamine utilization for cellular energy have been reported in several mammalian cells (Wellen et al., 2010). Together, the evidence implies that effective innate immune responses in the endometrium depend on glucose availability, glycolysis, and glutamine abundance.

INTRACELLULAR REGULATORS OF METABOLISM

As well as the dependence on the abundance of nutrients, cellular energy homeostasis is regulated by hormones and intracellular regulatory pathways. The major intracellular sensors of metabolic sufficiency are AMP-activated protein kinase (**AMPK**) and mammalian target of rapamycin (**mTOR**) (Hotamisligil and Erbay, 2008; Zoncu et al., 2011; Hardie et al., 2012). The principal regulator of cellular energy is AMPK, which senses the ratio of AMP:ATP in the cytosol (Fig. 2). As well as the metabolic energy required for lactation, demands on cellular energy to counter infections and repair tissues increase the ratio of

AMP:ATP, leading to activation of AMPK by phosphorylation (Hardie et al., 2012). Activated AMPK restores energy balance by inhibiting anabolic pathways that use ATP, whilst stimulating glycolysis, mitochondrial biogenesis, and increasing the expression of glucose and glutamine transporters. Mammalian target of rapamycin is a less specific sensor of cellular energy status than AMPK. The two main multi-protein mTOR complexes (**mTORC1** and **mTORC2**) integrate multiple signals of satiety from amino acid abundance, growth factors, hormones, and AMPK (Zoncu et al., 2011; Saxton and Sabatini, 2017). When nutrients are abundant, activation of mTOR stimulates anabolic pathways and cell growth. However, when cellular energy is limited, phosphorylated AMPK inhibits mTOR to conserve energy and limit protein synthesis.

Activating AMPK in endometrial organ cultures, using 5-Aminoimidazole-4-carboxamide ribonucleotide (**AICAR**) to mimic an energy deficit, reduced the secretion of IL-1β, IL-6 (Fig. 4A), and IL-8 in response to LPS by at least 50%, even in the presence of abundant glucose and glutamine (Turner et al., 2016). Surprisingly, inhibiting AMPK with Compound C, also reduced endometrial organ culture inflammatory responses to LPS (Turner et al., 2016). We interpreted these data to postulate that a homeostatic level of AMPK activation fosters optimal inflammatory responses.

In contrast to manipulation of AMPK, inhibition of mTOR to mimic cellular energy deficits had less impact on innate immunity. The mTOR inhibitor rapamycin had no significant effect on endometrial organ culture responses to LPS; whilst, the inhibitor Torin-1 gave a modest reduction in the secretion of IL-1β, IL-6 (Fig. 4B) and IL-8 (Turner et al., 2016). One explanation for these apparent discrepancies is that rapamycin inhibits mTORC1, whereas Torin 1 inhibits mTORC1 and mTORC2 (Zoncu et al., 2011; Saxton and Sabatini, 2017). Whilst mTORC2 may have a role in endometrial tissue under the conditions tested, the above data provide evidence that AMPK is an important link between cellular metabolic stress and innate immunity in the endometrium.

HORMONES

The pregnant cow has high peripheral plasma concentrations of progesterone and estradiol, which fall to basal levels after parturition, and postpartum dairy cows are often insulin-resistant and have lower plasma concentrations of IGF-1 (Beam and Butler, 1999; Wathes et al., 2009; LeBlanc, 2012; Wathes, 2012; Wathes et al., 2012; De Koster and Opsomer, 2013; Esposito et al., 2014). Metabolic stress in postpartum dairy cows can also limit luteinizing hormone pulse frequency, perturb the return of ovarian follicle activity, and delay ovulation (Beam and Butler, 1999). The impact of ovarian steroid hormones on postpartum uterine function is not clear. Progesterone is considered immuno-suppressive, whilst estradiol may enhance immunity and was used to treat uterine disease (Lewis, 2003). However, there are conflicting data, and exogenous estradiol infused into the bovine uterus during the postpartum period increased the abundance of bacteria in the uterus (Sheldon et al., 2004). Furthermore, the stage of the estrous cycle, or exogenous progesterone or estradiol, did not modulate innate immunity in ex vivo organ cultures of bovine endometrium (Saut et al., 2014). Similarly, treatment with estradiol or progesterone, or inhibitors of estradiol or progesterone nuclear receptors, did not affect gene or protein expression for IL-1β, IL-6 or IL-8 in endometrial cells or macrophages (Saut et al., 2014).

Apart from ovarian steroid hormones, the metabolic hormones insulin and IGF-1 are obvious candidates that might link postpartum metabolic stress and immunity, as concentrations of IGF-1 are reduced and animals are often insulin-resistant (Wathes et al., 2011; De Koster and Opsomer, 2013). Furthermore, IGF binding proteins and receptors have been characterized in the postpartum uterus, with differential expression between the previously gravid and non-gravid horns (Llewellyn et al., 2008; Wathes, 2012). However,

manipulating the concentration of exogenous IGF-1 did not affect the inflammatory response to LPS in ex vivo organ cultures of bovine endometrium (Turner et al., 2016). Other potential endocrine regulators of immunity for animals under postpartum stress might include growth hormone, leptin, prolactin, glucagon, ghrelin, prostaglandins and cortisol. Interestingly, cows that developed metritis had increased cortisol at parturition, compared with animals that had subclinical endometritis (Galvao et al., 2010). Furthermore, dexamethasone consistently reduces inflammatory responses to LPS in the endometrium and in immune cells (Saut et al., 2014). Whilst multiple hormones are important at the level of the whole animal, the molecular detail of how they impact tissue defenses is not yet clear.

CHOLESTEROL

When dairy cows mobilize adipose tissue to satisfy the negative energy balance of lactation, there is an increased peripheral plasma concentration of non-esterified fatty acids (Sordillo et al., 2009; Wathes et al., 2012). These fatty acids are metabolized in tissues to acetyl CoA to provide cellular energy, although excess fatty acid oxidation leads to increased production of ketones. During an immune response, tissue cells tend to further exploit fatty acid oxidation to supply nutrients, whereas immune cells exposed to pathogens often increase fatty acid synthesis as part of their inflammatory response (O'Neill et al., 2016). However, apart from cholesterol, there is limited information about the mechanistic cross-talk between lipids and immunity in the bovine endometrium.

Most of the cholesterol in cells is contained within the plasma membrane, and the concentrations of this lipid are tightly regulated by cholesterol uptake, cholesterol efflux transporters, and by cholesterol synthesis (Goldstein and Brown, 1990). The first steps in cholesterol synthesis are encompassed by the mevalonate pathway, converting acetyl-CoA to isoprenoids, which are then converted to squalene, and ultimately to cholesterol (Fig. 5). Cholesterol is partially responsible for the fluidity of plasma membranes, and as TLRs often dimerize within cholesterol-rich lipid rafts in membranes, changes in cellular cholesterol can impact innate immunity (Dykstra et al., 2003). Intriguingly, lower cholesterol concentrations in postpartum dairy cattle are also associated with uterine disease (Sepulveda-Varas et al., 2015).

To examine if the mevalonate pathway impacts innate immunity, chemical inhibitors and siRNA were used to target the mevalonate pathway enzymes, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (**HMGCoAR**), farnesyl diphosphate synthase (**FDPS**) and squalene synthase, which is also known as farnesyl-diphosphate farnesyltransferase 1 (**FDFT1**; Fig. 5). Whilst inhibiting HMGCoAR or FDPS had little effect on inflammation, inhibiting squalene synthase reduced IL-6 and IL-8 secretion in response to live *E. coli* or *T. pyogenes*, or LPS in endometrial tissue, and to LPS in epithelial and stromal cells (Fig. 4C) (Healey et al., 2016). As inhibition of squalene synthase might increase the abundance of the farnesyl and geranyl isoprenoids, and reduce the concentration of cholesterol (Fig. 5), these indirect effects were also examined (Healey et al., 2016). Although depletion of cholesterol with methyl-β cyclodextrin had no significant effect, treating endometrial cells with isoprenoids reduced interleukin secretion. These results provide evidence that the mevalonate pathway, and particularly inhibition of squalene synthase, can modulate innate immunity in the endometrium.

A discussion of the role of cholesterol in endometritis is incomplete without mentioning cellular resilience, because many bacteria secrete pore-forming toxins that target cholesterol in plasma membranes. In particular, *T. pyogenes* secretes pyolysin (**PLO**), which is a cholesterol-dependent cytolysin, and a key virulence factor associated with uterine disease in dairy cattle. Interestingly, PLO does not stimulate an inflammatory response but *T. pyogenes* does lead to inflammation, presumably via TLR2 on endometrial cells recognizing

bacterial lipopeptides (Borges et al., 2012; Turner et al., 2014). However, PLO causes considerable pathology by binding cholesterol-rich domains in the plasma membrane of endometrial cells and forming pores that lead to osmotic cell death (Amos et al., 2014; Preta et al., 2015). Endometrial stromal cells have abundant cholesterol and are particularly sensitive to PLO, which may explain how *T. pyogenes* switches from a commensal bacterium when the epithelium is intact, to cause uterine pathology once the epithelium is breached during and after parturition, allowing bacteria to access the stroma (Amos et al., 2014). Reducing cellular cholesterol, using methyl-β-cyclodextrin or an endocytosis inhibitor, increases the resilience of stromal cells to PLO (Amos et al., 2014; Preta et al., 2015). Taking the observations together, reducing cellular cholesterol may limit inflammation, but increases stromal cell resilience to pore-forming toxins.

CONCLUSION

The present review starts with the premise that metabolic stress, particularly negative energy balance, is associated with postpartum metritis and endometritis. Resisting bacterial infections depends on the prompt detection of pathogens by the innate immune system, followed by rapid and robust inflammatory responses. Here we focused on mechanisms in endometrial tissues and cells. We describe several mechanisms where cellular metabolic stress may limit the necessary acute inflammatory response, and the prompt resolution of bacterial infections. The strongest links between metabolism and immunity appear to reflect the coevolution of cellular metabolism and innate immunity. In particular, inflammatory responses are dependent on the abundance of glucose and glutamine for cellular energy, and the role of AMPK, which is the principal sensor of ATP sufficiency. Whereas, the more generalized regulators of cell metabolism, including mTOR and IGF-1, appear to have a lesser impact on inflammation. The interrelationship between disease and metabolism are highly complex, as illustrated by cholesterol homeostasis, which can impact both innate immune responses to pathogen-associated molecules, and cellular resilience to pore-forming toxins. The system in vivo is even more complex, and so insights from the endometrium are only a small part of the whole story. However, the consistent feature amongst the evidence for links between cellular metabolism and immunity is that metabolic homeostasis fosters optimal inflammatory responses to resist pathogens. We suggest that stressing cellular metabolism increases the risk of uterine disease by impairing endometrial defenses.

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FIGURE LEGENDS

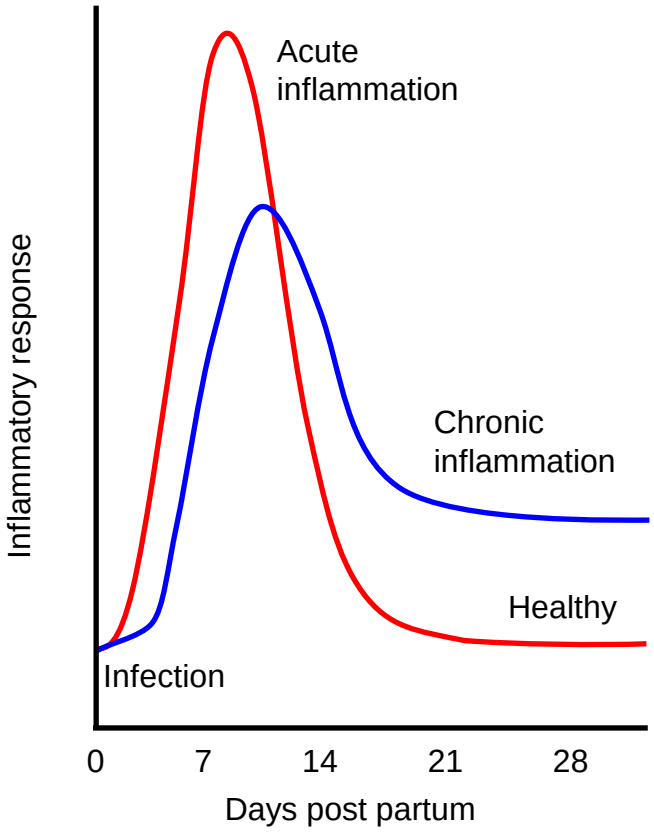
Figure 1. Progression of inflammation during bacterial infection. Rapid and robust acute inflammatory responses (red line) are important to resist bacterial infections effectively. Once the infection is controlled, there is a return to homeostasis by active resolution of inflammation and repair of damaged tissues. Delayed or inadequate inflammatory responses may not fully resolve infections (blue line), leading to chronic inflammation.

Figure 2. Cellular energy metabolism. Most ATP for cellular energy is generated by glucose flux through glycolysis into the Krebs cycle, followed by oxidative phosphorylation in mitochondria. Infections also induce the Warburg effect, with increased glucose flux through glycolysis and conversion of pyruvate to lactate by lactate dehydrogenase (LDH), leading to the rapid generation of ATP and substrates for membranes, nucleotides and proteins. Glutamine also contributes to ATP production by entering the Krebs cycle after glutaminolysis by glutaminase (GLS) and conversion to α-ketoglutarate. As well as entering the Krebs cycle, α-ketoglutarate can be converted to citrate in mitochondria and the cytoplasm by isocitrate dehydrogenase (IDH), and citrate contributes to lipid synthesis. The intracellular sensors AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) regulate cellular metabolism.

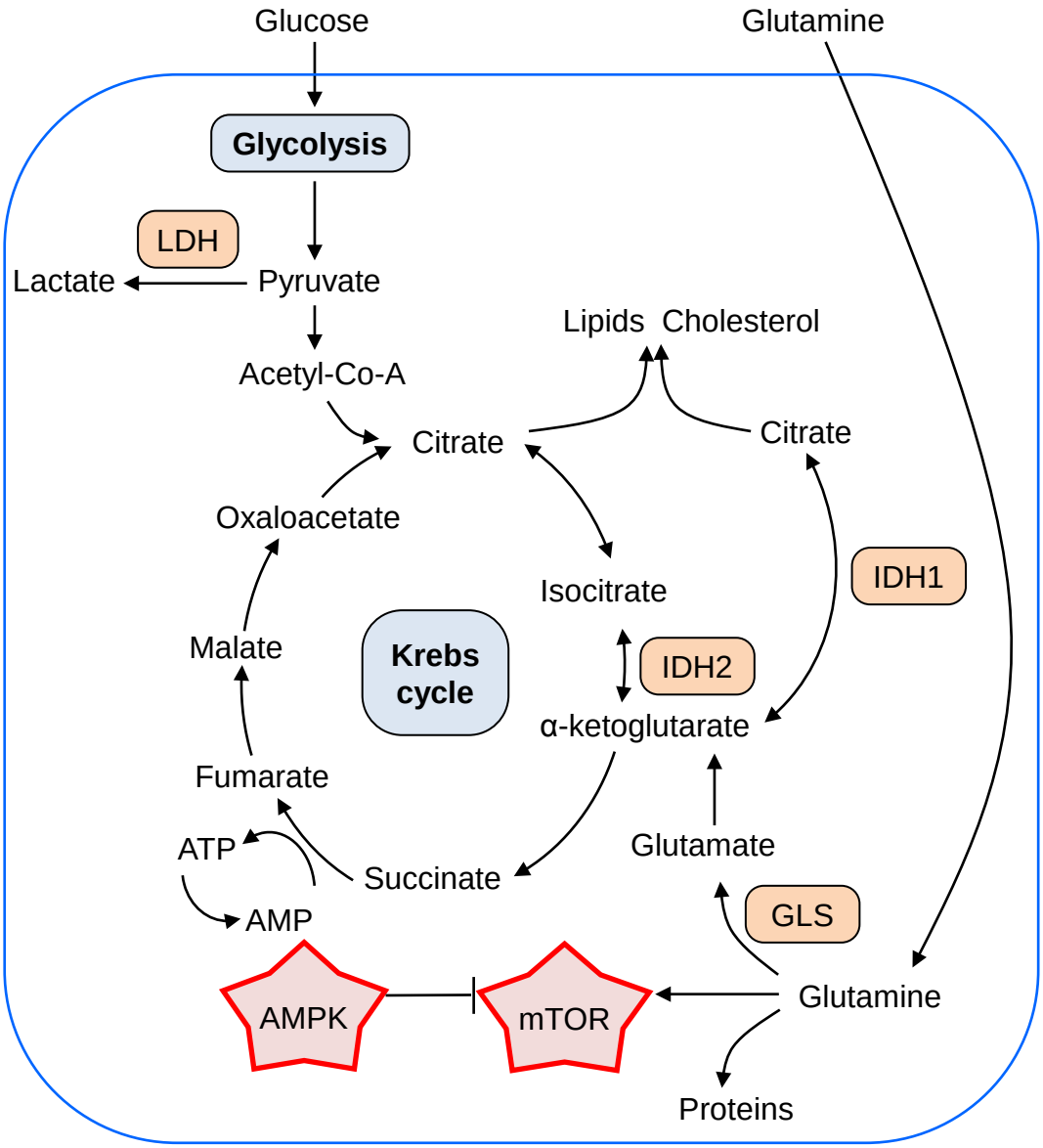
Figure 3. Glucose and glutamine availability modulates inflammation in endometrial tissue. Ex vivo organ cultures of bovine endometrium were cultured for 24 h in media containing a range of amounts of glucose (A, B) or glutamine (C, D), and then challenged with control vehicle (○) or 100 ng/ml lipopolysaccharide (LPS, ■) for a further 24 h. Limiting the abundance of glucose or glutamine reduced ($P < 0.05$, ANOVA) the accumulation of the pro-inflammatory cytokines IL-1β (A,C) and IL-6 (B,D) in response to LPS. Graphs are redrawn from Turner et al., 2016 and Noleto et al., 2017, and present mean + SEM concentration per mg endometrial tissue.

Figure 4. Metabolic regulators in cells impact inflammation. Ex vivo organ cultures of endometrium were cultured for 24 h in medium containing the indicated amounts of (A) AICAR (5-Aminoimidazole-4-carboxamide ribonucleotide) to activate AMP-activated protein kinase, or (B) Torin 1 to inhibit mammalian target of rapamycin. (C) Bovine endometrial stromal cells were cultured for 24 h in medium containing the indicated amounts of squalestatin to inhibit squalene synthase. Cultures were then treated with control vehicle (○) or 100 ng/ml lipopolysaccharide (LPS, ■) for a further 24 h. Each of the compounds reduced ($P < 0.05$, ANOVA) the accumulation of IL-6 in response to LPS. Graphs are redrawn from Turner et al., 2016 and Healey et al., 2016, and present mean + SEM concentrations per mg endometrial tissue (A, B), or per ml of culture supernatant (C).

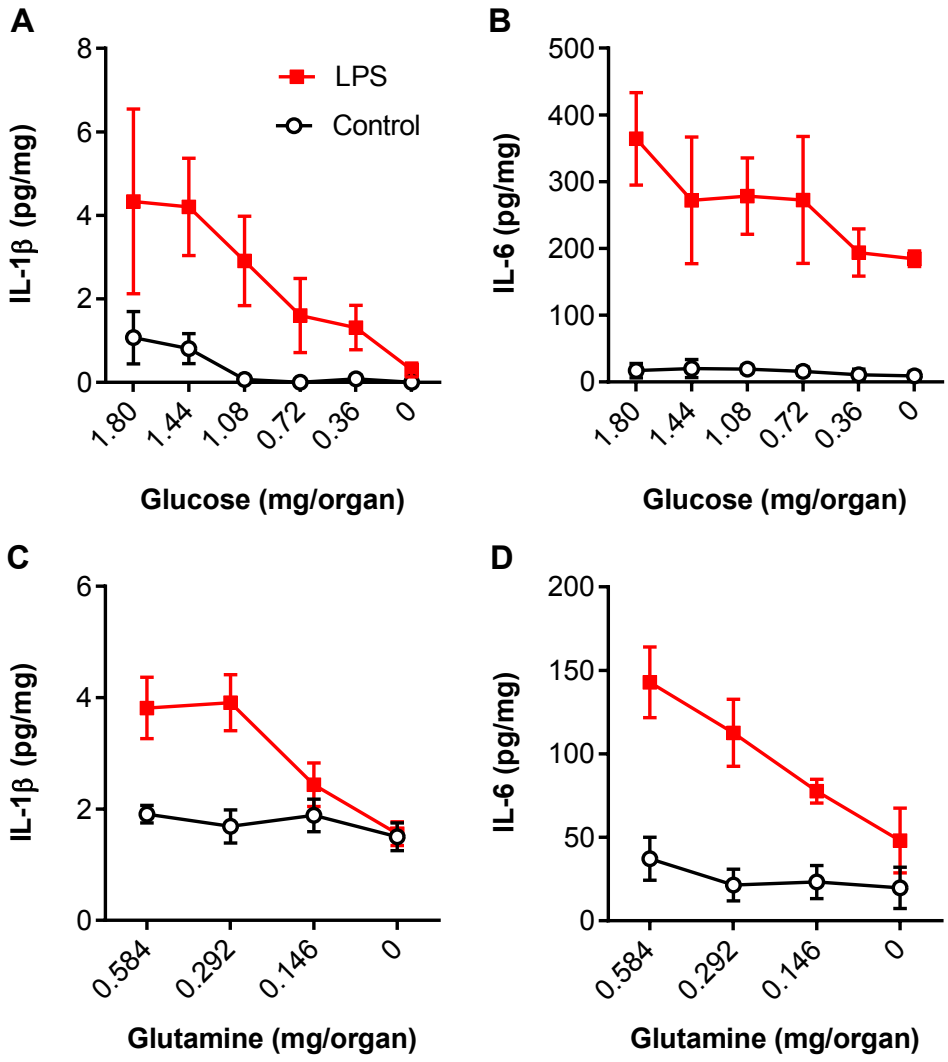
Figure 5. The mevalonate pathway and cholesterol synthesis. Acetyl CoA and acetoacetyl CoA are converted via the isoprenoids to squalene, which is subsequently converted to cholesterol. The key enzymes in the mevalonate pathway are 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCoAR), farnesyl diphosphate synthase (FDPS), and squalene synthase (SQS). Each of these enzymes can be inhibited by statins, bisphosphonates, and squalestatin, respectively; and, cellular cholesterol can be depleted using methyl-β-cyclodextrin.



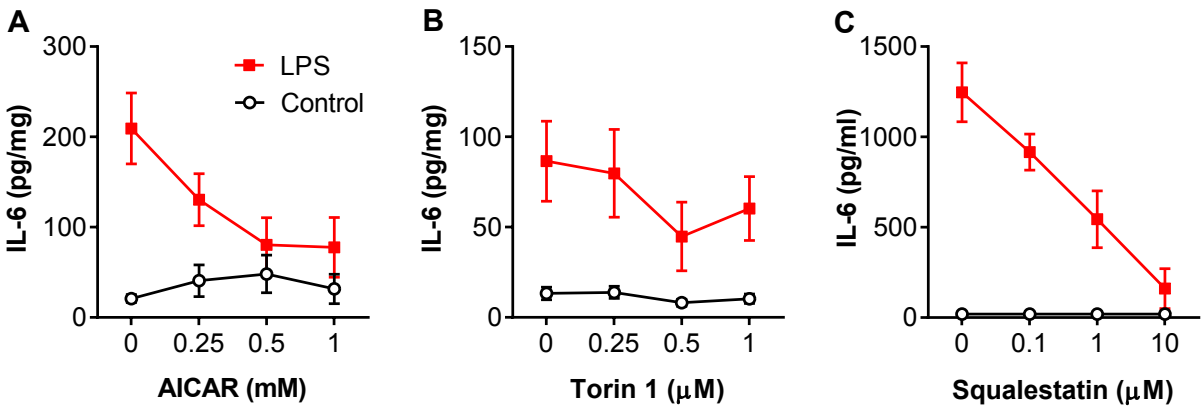
JDS-17-13135 Figure 1



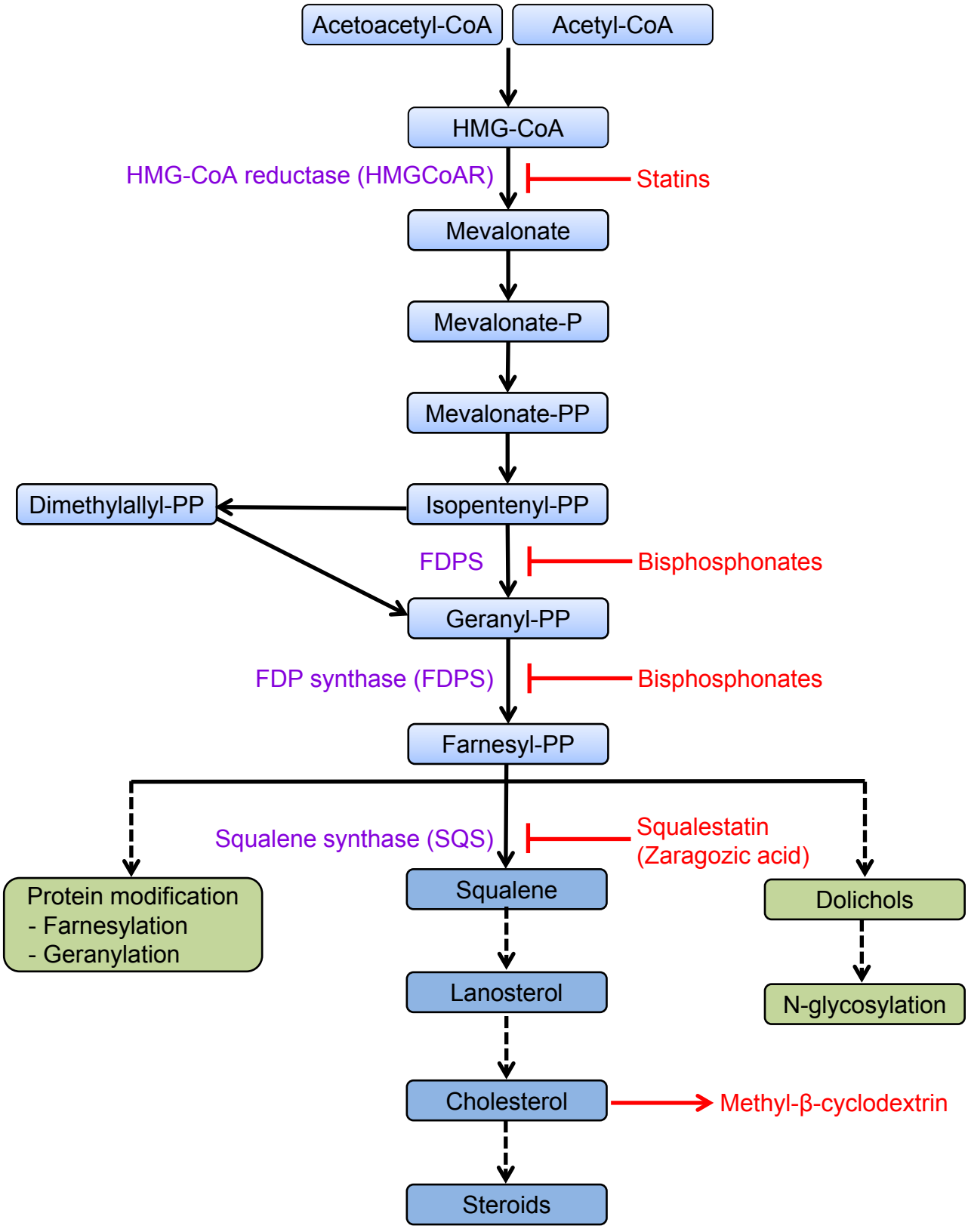
JDS-17-13135 Figure 2



JDS-17-13135 Figure 3



JDS-17-13135 Figure 4


Acetoacetyl-CoA
Acetyl-CoA
HMG-CoA
HMG-CoA reductase (HMGCoAR)
Statins
Mevalonate
Mevalonate-P
Mevalonate-PP
Dimethylallyl-PP
Isopentenyl-PP
FDPS
Bisphosphonates
Geranyl-PP
FDP synthase (FDPS)
Bisphosphonates
Farnesyl-PP
Squalene synthase (SQS)
Squalestatin
(Zaragozic acid)
Protein modification
- Farnesylation
- Geranylation
Squalene
Dolichols
Lanosterol
N-glycosylation
Cholesterol
Methyl-β-cyclodextrin
Steroids