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Boots for Achilles

Progesterone's Reduction of Cholesterol is a Second-Order Adaptation

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Abstract

Progesterone and cholesterol are both vital to pregnancy. Among other functions, progesterone downregulates inflammatory responses, allowing for maternal immune tolerance of the fetal allograft. Cholesterol, a key component of cell membranes, is important in intracellular transport, cell signaling, nerve conduction, and metabolism. Despite the importance of each substance in pregnancy, one exercises an antagonistic effect on the other, as periods of peak progesterone correspond with reductions in cholesterol availability, a consequence of progesterone's negative effects on cholesterol biosynthesis. This arrangement is understandable in light of the threat posed by pathogens early in pregnancy. Progesterone-induced immunomodulation entails increased vulnerability to infection, an acute problem in the first trimester, when fetal development is highly susceptible to insult. Many pathogens rely on cholesterol for cell entry, egress, and replication. Progesterone's antagonistic effects on cholesterol thus partially compensate for the costs entailed by progesterone-induced immunomodulation. Among pathogens to which the host's vulnerability is increased by progesterone's effects, approximately 90% utilize cholesterol, and this is notably true of pathogens that pose a risk during pregnancy. In addition to having a number of possible clinical applications, our approach highlights the potential importance of second-order adaptations, themselves a consequence of the lack of teleology in evolutionary processes.

Keywords: progesterone, cholesterol, pregnancy, infection, evolutionary medicine

Introduction

Cholesterol and progesterone each play a vital role in pregnancy. Intriguingly, despite the importance of each, the latter exercises an antagonistic effect on the availability of the former. Here, we argue that this puzzling arrangement reflects an evolved second-order adaptation, that is, an adaptation that addresses an adaptive challenge that is itself a consequence of the effects of another adaptation. Consider the following: It is well documented that cholesterol plays an important role in fetal development (Brizzi et al. 1999; Innis 2005; Woollett 2011). Although maternal triglycerides cannot directly cross the placental membrane, free fatty acids and ketones produced from those trigylcerides can cross the barrier, and are utilized by the fetus as both fuels and lipogenic substrates (Brizzi et al. 1999). It has been shown that when maternal plasma cholesterol is low (<160 mg/dl), birthweights are lower than normal, and there is a trend for microcephaly (Edison et al. 2007), suggesting that, although the fetus can also metabolize its own cholesterol, its capacity in this regard is limited, and hence inadequate transfer of maternal cholesterol components negatively impacts fetal growth (Woolett 2011). Correspondingly, low intakes of specific fatty acids by the mother during gestation can result in decreased neural growth cones in the fetal brain, liver, and the placenta (Innis 2005). Paralleling the importance of cholesterol, the ovarian hormone progesterone, vital for the success of pregnancy, is unequivocally required in all mammals for maternal support of conceptus survival and development (Spencer and Bazer 2002). Progesterone is essential to several important events in the establishment of pregnancy, including ovum transport, endometrial cell proliferation, differentiation, decidualization, and the process of implantation. It is also vital for the maintenance of pregnancy, and a loss of progesterone is causally associated

with miscarriage in early pregnancy (Macdonald 1989). It is therefore striking that a characteristic action of progesterone is its reduction of cholesterol. Progesterone has been shown to inhibit the esterification of cholesterol derived from low-density lipoproteins (LDLs), preventing its delivery to cellular enzymes (Metherall et al. 1996). Treatment of cholesterol with progesterone also causes the accumulation of sterol precursors, implying that cholesterol production pathways are disrupted (Lindenhall et al. 2001). Indeed, progesterone disrupts pathways involved in both cholesterol biosynthesis and the processing of LDL-derived cholesterol (Lindenhall et al. 2001).

Why would a hormone that is intimately linked to successful pregnancy cause a reduction in the availability of a building block needed for fetal development? While seemingly paradoxical, we suggest that this relationship constitutes a compromise solution to a problem that arises due to tradeoffs inherent in pregnancy. Below we review the respective roles of cholesterol and progesterone, describe the effects of progesterone on cholesterol availability, delineate the potential costs of this interaction, and then outline the benefits, in the form of reduced vulnerability to pathogens, that we hypothesize outweigh these costs.

The functions of cholesterol and its role in fetal development

Cholesterol, a lipid molecule with a characteristic four-ring steroid structure (Yoshida and Yoshinao 2005), is vital to life. It is required as the structural component of mammalian cell membranes, helping to maintain proper permeability and fluidity (Yeagle 1985). Cholesterol also functions in intracellular transport, cell signaling, (Maxfield and Tabas 2005) and nerve conduction (Saher et al. 2011). It is a fundamental mediator of

metabolism through the propagation of signaling cascades, and is essential to both the activation and propagation of hedgehog signaling (Woollett 2005). Additionally, cholesterol is the precursor for other necessary compounds, such as steroid hormones, bile acids, and Vitamin D (Lecerf et al. 2011), and is a significant component of lipid rafts in the plasma membrane, which serve as organizing centers for the assembly of signaling molecules (Rosenberger et al. 2000). Lipid rafts themselves play multiple functions, including polarized secretion, membrane transport, transcytosis across epithelial monolayers, and the generation of cell polarity (Rosenberger et al. 2000). Two types of lipoproteins are associated with cholesterol: high-density lipoproteins (HDLs) and low-density lipoproteins (LDLs). These function in the transport of cholesterol throughout the body. LDLs assist in the transport of cholesterol out of the liver, while HDLs act as acceptors of cholesterol, and are believed to bring fat and cholesterol back to the liver (Assmann and Gotto 2004; Grummer and Carroll 1988). Regulation of synthesis, influx, and effux keeps cellular cholesterol levels tightly controlled (Simons and Ikonen 2000).

Reflecting its many important functions in the body, cholesterol is a vital factor in development. Cholesterol's relationship to the Sonic hedgehog (Shh) group of proteins entails an essential role in embryonic development, as these proteins are required for morphogenesis; cholesterol modulates the function of the Shh group by binding a functional Shh fragment and thereby restricting the distribution and activity of the Shh signal on the cell membrane (Yoshida and Yoshinao 2005). Correspondingly, cholesterol deficits during embryogenesis cause severe abnormalities (Kolejakova et al. 2010). To take one example, Smith-Lemil-Opitz syndrome, caused by an inherited defect in a specific enzyme in the cholesterol biosynthesis pathway, is characterized by abnormal

development and poor function, especially in cognition (Salen et al. 1996). This is further supported by evidence showing correlations between statin use in pregnancy and fetal neurological damage, and impaired placental implantation and function (Kenis et al. 2005; Lockshin 2010; Pollack et al. 2005).

Consonant with the above, reflecting the substantial need for cholesterol during this time of rapid growth, fetal sterol synthesis rates are greater than those in other extrahepatic tissues (Woollett 2005). Importantly, however, although the fetus is able to synthesize its own cholesterol, because demand generally outstrips supply -- such that maternal contribution is a limiting factor in fetal growth (Gluckman and Hanson 2004) -- the fetus is dependent on maternal supply. Maternal cholesterol, in the form of lipoproteins, can enter into fetal circulation through uptake by the placenta and trophoblasts, via both receptor-mediated and receptor-independent transport (Woollett 2005). Correspondingly, studies consistently reveal an intimate relationship between maternal cholesterol levels and healthy fetal development (Brizzi et al. 1999; Innis 2005; Woollett 2011).

Progesterone-mediated reductions in cholesterol during pregnancy, the luteal phase, and menopause

Early pregnancy is associated with a nadir in the mean value of serum cholesterol (Darmady and Postle 1982; Basaran 2009). Although cholesterol rises steadily through gestation, early in the first trimester there is an initial decrease in plasma lipids (Basaran 2009; Sep et al. 2011). Cholesterol levels eventually climb dramatically, but recovery from the initial decline is gradual, such that LDL levels at the end of the first trimester are often

still within normal ranges (Brizzi et al. 1999); it is generally only by the beginning of the second trimester that cholesterol levels rise substantially above the pre-pregnancy baseline (Basaran 2009).

Importantly, the first-trimester decline in maternal cholesterol levels is not a consequence of utilization of maternal cholesterol by the conceptus, as total conceptus cell mass is small during the first-trimester cholesterol nadir, and, moreover, the general pattern of cholesterol decline is not dependent on conception, as it also occurs during the luteal phase of menstrual cycles in which conception has not taken place. During the follicular phase of the menstrual cycle, total cholesterol levels peak (Ahumada-Hemer et al. 1985; Kim and Kalkhof 1979; Jones et al. 1988), as do levels of LDL (Ahumada-Hemer et al. 1985; Tikkanen et al. 1986). During the luteal phase, in which the endometrium is prepared for implantation, levels of both total serum cholesterol and triglycerides decline (De Leon et al. 1992). The luteal phase constitutes preparation for pregnancy, and, correspondingly, pregnancy can be conceptualized as a continuation of changes present mid-luteally – declines in cholesterol thus occur in anticipation of, rather than as a consequence of, the presence and growth of a conceptus.

Progesterone is the principal candidate for the cause of the decline in cholesterol in the luteal phase and the early first trimester. Progesterone remains at a relatively low level throughout the follicular phase and during ovulation, but increases sharply during the luteal phase (De Leon et al. 1992). In the event of conception and implantation, progesterone continues to climb across the first trimester (Tay and Lenton 2002). Importantly, progesterone's cholesterol-reducing effects are well established. Studies have shown that progesterone inhibits the delivery of LDL-derived cholesterol to processing

enzymes such as Acetyl-Coenzyme A acetyltranferase (ACAT) (Metherall et al. 1996). Progesterone inhibits the movement of LDL-derived cholesterol from lysosomes to the plasma membrane (Plemenitas et al. 1990), and the movement of cholesterol from the plasma membrane to the endoplasmic reticulum (Lange 1994). This movement of sterols from the plasma membrane to the endoplasmic reticulum is required for cholesterol biosynthesis (Metherall et al. 1996); thus, progesterone's impediment of LDL-derived cholesterol movement in turn impedes cholesterol biosynthesis. Consistent with the disruption of cholesterol production pathways, treating tissue with progesterone leads to an accumulation of sterol precursors (Lindenhall et al. 2001). At the organismic level, exogenous progesterone has been shown to reduce HDL cholesterol both when administered through progestin-only oral contraceptives (Wynn and Niththyananthan 1982) and when administered through hormone-replacement therapies in post-menopausal women (Lamon-Fava et al. 2006).

Menopause is accompanied by a dramatic decline in progesterone levels and, consistent with the above portrait, across diverse populations, there is a corresponding increase in serum cholesterol during this period independent of the effects of age (Wu et al. 1990; Akahoshi et al., 1996; Mathews et al., 2009); correspondingly, surgical menopause has a similar effect (Akahoshi et al., 1996).

We are thus faced with the apparent contradiction that a hormone that is central to pregnancy causes a reduction in lipids that, being vital to cellular activity and cell division, are crucial to successful fetal development. To date, this question has not been explored. Several authors (Butte 2000; Toescu et al. 2002) have noted in passing that low cholesterol levels early in pregnancy correspond to an anabolic phase during which fat deposition is

187 enhanced in anticipation of late pregnancy, when rapid fetal growth will require maternal
188 catabolism. In this view, the initial reduction in gestational cholesterol levels is simply a
189 side-effect of the need to lay in energy stores for later. However, the ratio of cholesterol to
190 triglycerides in fat cells is both constant and largely independent of cell size, indicating that
191 both are likely deposited simultaneously in a fixed ratio (Kovanen et al. 1975) – a feature
192 inconsistent with progesterone's disruption of cholesterol production. Hence, while there
193 is conclusive evidence of a patterned shift from anabolism to catabolism across pregnancy,
194 this pattern provides neither proximate nor ultimate explanations of the antagonistic
195 effects of progesterone on cholesterol synthesis. Rather, we propose that the solution to
196 this puzzle lies in the intersection of the effects of progesterone on the immune system and
197 the role of cholesterol in infection.
198
199 **Progesterone-induced immunomodulation and compensatory prophylaxis**
200 With half of its genome being paternally derived, from the perspective of the
201 maternal immune system, the conceptus constitutes a genetically incompatible allograft.
202 As a consequence, changes must occur in the maternal immune system in order to prevent
203 maternal lymphocytes from attacking the conceptus (Szekeres-Bartho et al. 1983).
204 Pregnancy is facilitated by a shift in the Th1/Th2 balance in maternal immune functioning,
205 a move away from those inflammatory responses that pose the greatest danger to the
206 invasive blastocyst and the subsequently semi-parasitic embryo (reviewed in Fessler 2002;
207 Doyle et al. 2007; and Fleischman and Fessler 2011). Importantly, progesterone plays a
208 central role in the immunomodulation necessary to tolerate the half-foreign conceptus
209 (Siiteri et al. 1977). The downregulation of maternal inflammation is achieved through

decreased levels of pro-inflammatory cytokines and natural killer cells. These changes are the downstream consequence of progesterone-induced blocking factor (PIBF), which shifts the maternal immunological balance toward anti-inflammatory signals (reviewed in Fessler 2001; see also Szekeres-Bartho et al. 1995; Doyle et al. 2007). Hence, progesterone is essential to pregnancy in part because it commands an immunomodulatory cascade that allows for tolerance of the half-foreign parasitic conceptus.

Maternal immune tolerance of the conceptus comes at a price, as, by lowering host defenses, it increases the chances of infection (reviewed in Fessler 2001, Fessler 2002, and Doyle et al. 2007). PIBF alters the cytokine secretion profile by increasing the production of Th2 cytokines and decreasing the production of Th1 cytokines (Faust et al. 1999). PIBF has also been shown to inhibit natural killer cell activity, through a blockade of degranulation (Faust et al. 1999). Both of these changes increase vulnerability to infection by lowering defenses in regard to both the detection and elimination of pathogens. Indeed, some pathogens may have evolved the ability to exploit this temporary weakening of host defenses; for example, progesterone not only increases the probability of infection by cytomegalovirus, but, moreover, actually increases the pathogen's virulence (Chong and Mims 1984). Furthermore, increased maternal susceptibility to infection comes at a particularly dangerous time, as a) later in pregnancy the fetus eventually develops some autonomous defenses against pathogens, but these are absent early in development (Holt and Jones 2000), and b) the early first trimester is a critical period in fetal development, as organogenesis, concentrated during this phase, is a process that is especially vulnerable to insult (Arnold 1990), and, correspondingly, infection during the first trimester often can have drastic consequences (Wright 1966).

233 Progesterone's effects on the immune system clearly constitute an adaptation that
234 serves to allow for gestation. Yet, this adaptation comes at the cost of increased
235 susceptibility to infection during a particularly vulnerable period. Importantly, natural
236 selection is not a teleological process – innovations that solve one problem can create
237 another. Moreover, the liabilities entailed by one trait can, in turn, constitute a source of
238 selective pressure leading to the evolution of new traits that mitigate the costs of these
239 liabilities. Such second-order adaptations have been variously referred to as adaptive
240 workarounds (Eastwick 2009) or the product of compensatory mutations (Maisnier-Patin
241 and Andersson 2004). Of relevance to the matter at hand, recent evidence suggests that
242 changes in other systems adaptively mitigate the vulnerability to pathogens entailed by
243 progesterone's effects on the immune system. Specifically, alterations in behavior provide
244 one avenue for such mitigation. The compensatory prophylaxis hypothesis holds that,
245 because prophylactic behavior entails time, energy, attention, and opportunity costs, rather
246 than remaining constant, prophylactic behavior should be enhanced during periods of
247 increased susceptibility to infection, when the greater benefits obtained merit increased
248 expenditures (Fessler and Navarrete 2003). Hence, because progesterone regulates a
249 cascade of physiological events that result in increased vulnerability to infection,
250 progesterone can be expected to also mediate increased behavioral prophylaxis (Fessler
251 and Navarrete 2003). Consonant with this hypothesis, studies have shown increases in
252 disgust sensitivity, a proximate mechanism subserving disease avoidance, during the
253 vulnerable first trimester (Fessler et al. 2005). More specifically, disgust sensitivity,
254 disease-avoidance behaviors, and related perceptions and attitudes all increase as a
255 function of progesterone levels (Fleischman and Fessler 2011; Navarete et al. 2007;

256 Conway et al. 2007; but see also Fessler and Navarrete 2003). Likewise, preferences for
257 healthy over unhealthy faces (a cue of disease risk) are elevated during periods of elevated
258 progesterone (Jones et al. 2005). Although compensatory prophylaxis is behavioral, we
259 believe that a similar logic explains the effects of progesterone on cholesterol, as the latter
260 plays a key role in infection.
261
262 **Cholesterol and infection**
263 Critically, cholesterol plays a key role in infection. Lipid rafts are sites of entry and
264 exit for a wide variety of viruses (Medigeshi et al. 2008). Lipid rafts can be exploited by
265 pathogens in a number of ways. Some viruses, such as human immunodeficiency virus type
266 1, coxsackievirus, simian virus 40, and severe acute respiratory syndrome coronavirus,
267 depend on lipid rafts for binding to and entry into the host cell; other viruses, such as
268 rotavirus, Newcastle disease virus, influenza virus, Ebola virus, and Marburg virus, utilize
269 raft-mediated pathways for assembly and egress (Chazal and Gerlier 2003; Manes et al.
270 2003; Ono and Freed 2005; Pelkmans 2005).
271 A number of bacteria similarly exhibit cholesterol dependence, including *Anaplasma*
272 *phagocytophilium* (Xiong et al. 2009), *Escherichia coli* (Goluszko and Nowicki 2005),
273 *Mycobacterium* (Gatfield and Pieters 2000), *Staphylococcus aureus* (Liu et al. 2008),
274 *Salmonella* (Hayward et al. 2005), and *Shigella* (Hayward et al. 2005), among others. Some,
275 such as *Mycobacterium tuberculosis*, utilize cholesterol as a primary carbon source
276 throughout the course of infection, such that degradation of this sterol is crucial for
277 bacterial persistence (Miner et al. 2009). In other cases, in species such as *Staphylococcus*
278 *aureus* that do not use cholesterol as a significant energy source (Shine et al. 1993),

disruption of cholesterol biosynthesis nevertheless blocks bacterial virulence (Liu et al. 2008), as cholesterol is a key component of the cytoplasmic membrane (Yeagle 1985). Cholesterol dependency can be a distinguishing feature of the pathogenic adaptations of bacteria; indeed, an entire family of bacterial cytolysins is referred to as cholesterol-dependent cytolysins (CDCs) because they can only function effectively in the presence of host cholesterol. These pore-forming toxins are produced by more than twenty species from the genera *Clostridium, Streptococcus, Listeria, Bacillus*, and *Arcanobacterium* (Tweten 2005). Cholesterol-dependent cytolysins function both as simple hemolysins and as general cell-lytic agents that are crucial in bacterial infection (Tweten 2005). Conversely, bacterial sepsis causes decreases in the concentrations of total cholesterol, HDL, and apoproteins A and B of patients; the return of serum lipids to more normal concentrations parallels the recovery from sepsis (Alvarez and Ramos 1986). Although at present there is no consensus as to why this correlation exists, it may be that hypocholesterolemia in cases of sepsis is a component of defensive responses (Das et al. 2011).

Consonant with the thesis that the availability of cholesterol is a determinant of the ability of pathogens to proliferate, intriguing indications are emerging of a relationship between statin therapy, which decreases cholesterol, and a lower incidence of severe sepsis (Almog 2003). Statins display antimicrobial effects in many studies. Both in vivo and in vitro, statins reduce the intracellular growth of a subspecies of *Salmonella enterica* (Catron et al. 2004), while simvastatin has shown a significant antimicrobial effect against MSSA, and, to a lesser extent, against MRSA (Jerwood and Cohen 2008).

Given that cholesterol plays a role in infectious disease, it is tempting to ask whether epidemiological studies reveal a link between cholesterol and infection. However, before

reviewing this evidence, it is important to note that it is difficult to predict in advance how such correlations will play out. On the one hand, pathogens' dependence on cholesterol suggests that we might expect a straightforward positive correlation between the host's systemic cholesterol levels and morbidity and mortality due to infection. On the other hand, however, if the body is able to facultatively adjust cholesterol levels as a function of the individual's capacity to resist infection, then the opposite pattern may obtain, as individuals who are vulnerable to pathogens for reasons other than cholesterol availability may both exhibit lower cholesterol levels (reflecting an attempt to reduce vulnerability) and suffer higher rates of morbidity and mortality due to infection (reflecting the incomplete success of such efforts). Lastly, complicating the picture still further, it may be important to distinguish between different affordances of cholesterol from the perspective of the host. Although cholesterol facilitates infection and pathogen proliferation, once infection is established, cholesterol may sometimes benefit the host by reducing the destructive effects of endotoxins produced by some bacterial pathogens (Ravnskov 2003; Feingold et al. 1995). Accordingly, among individuals who are able to mount a robust immune response to infection, those having high cholesterol levels may suffer less pathogen-driven morbidity and mortality than those having low cholesterol levels.

Hospital studies reveal that low levels of HDL increase the probability of nosocomial infections (Canturk et al. 2002; Delgado-Rodriguez et al. 1997), and are predictors of in-hospital death and length of stay (Delgado-Rodriguez et al. 2002). These patterns are consistent with the thesis that cholesterol availability directly determines risk of infection, as HDL functions to transport excess cholesterol from the periphery to the liver for excretion into bile (Zhang 2003), hence lower HDL levels equate to less reverse cholesterol

transport and organ clearance, which, in turn, could conceivably lead to an increase in the amount of cholesterol available to pathogens elsewhere in the body. Conversely, however, outside of the hospital, among men, total cholesterol is inversely related to urinary tract, venereal, musco-skeletal, and all infections, and, among women, to urinary tract, all genito-urinary, septicaemia, bacteraemia, miscellaneous viral site unspecified, and all infections (Iribarren et al. 1998). Given the role of cholesterol in infection, the latter pattern strongly suggests that individual differences in cholesterol levels may reflect underlying differences in immunologic robustness, such that more vulnerable individuals maintain lower cholesterol levels in an incompletely successful effort to compensate for their vulnerability to parthogens.

A less direct route to exploring the relationship between cholesterol levels and infection is to consider cholesterol's effects on overall mortality. One difficulty, however, in interpreting such patterns is the question of how to evaluate the respective effects of cholesterol on susceptibility to infection and cardiovascular disease. Although conventional wisdom holds that cholesterol contributes directly to cardiovascular disease, consonant with the view advanced here, Ewald (2008) presents a strong case that this correlation actually reflects the role of cholesterol in facilitating infection by pathogens such as Chlamydia that, in turn, damage blood vessels. Nevertheless, given that this remains a minority view, it is conservative to evaluate the contributions of cholesterol to mortality independent of deaths due to cardiovascular disease. Although a number of studies have sought to elucidate the relationship between cholesterol and non-cardiovascular mortality, at present there is no consensus in the literature in this regard. Age may be an important factor. In adults older than 85 years, high total cholesterol

concentrations are associated with longevity, seemingly from lower mortality due to cancer and infection (Weverling-Rijnsburger et al. 1997). A similar pattern of the protective effects of cholesterol has also been found among adults older than 55, who evince an inverse relationship between total cholesterol and several infectious diseases (Iribarren et al. 1998). Conversely, studies of younger adults reveal that the effect of total cholesterol on non-cardiovascular mortality is neutral (Kronmal et al. 1993; Krumholz et al. 1994; Gould et al. 1995). Another study finds a trend of increased non-cardiovascular mortality with decreased LDL, in both placebo and treatment groups (Razzolini et al. 2008). However, at each given LDL cholesterol level, non-cardiovascular mortality is lower in patients treated with statins (Razzolini et al. 2008).

To summarize the above, at the cellular level, there is substantial evidence that cholesterol can play a key role in infection. At the population level, the picture is more mixed, possibly reflecting both complex relationships between cholesterol levels and immunological robustness and the effects of cholesterol on other aspects of health. Here, we are concerned with the possibility that patterned changes in systemic cholesterol can adaptively mitigate vulnerability to infection entailed by progesterone's effects on the immune system. Given the extent to which pathogens are dependent on cholesterol, if humans have indeed evolved mechanisms capable of such compensatory adjustment, then we should expect to find evidence of an evolutionary arms race between human hosts and a variety of pathogens, as each seeks to gain control of cholesterol availability in order to determine the outcome of infection.

Arms races between host and pathogens over cholesterol regulation/synthesis

There appears to be a correlation between innate immune signaling processes and the regulation of sterol metabolism (Castrillo et al. 2003; Zelcer and Tontonoz 2006; Ogawa et al. 2005; Wang et al. 2009). In keeping with the role of cholesterol in infection and the corresponding strategic value of its regulation, a relationship has been demonstrated between the cholesterol-metabolic pathway and protection against, or susceptibility to, infection (Blanc et al. 2011). Specifically, mammalian hosts produce high levels of interferons after infection with a range of viruses; in turn, via interferon receptors, high levels of interferons lower enzyme levels on the cholesterol pathway, resulting in a net reduction in cholesterol availability (Blanc et al. 2011). Host reduction of cholesterol as a defense mechanism is also observed in conjunction with the hepatitis C virus (HCV) (Walters et al. 2006). However, in keeping with the advantages to the pathogen of cholesterol abundance, HCV counters this move by impairing lipid metabolism and causing an unregulated increase in cholesterol and fatty acid synthesis (Nakamuta et al. 2009). In response, infected cells catalyze rate-limiting steps in the cholesterol pathway to reduce the amount of cholesterol produced, while increased expression of genes associated with peroxisomes, which are capable of breaking down cholesterol, suggests attempts to prevent the pathogen from utilizing previously produced cholesterol (Walters et al. 2006). Thus, it appears that the host is engaged in an arms race with HCV to regulate the production and availability of cholesterol. The same is likely true of other pathogens as well, as, in a variety of viral pathogens, there is a correlation between increased virulence and increase in fatty acid supply and synthesis. Human cytomegalovirus has been shown to alter fatty acid biosynthesis pathways to increase fatty acid supply, which is essential for optimal viral growth (Munger et al. 2008). West Nile virus acts similarly, modulating host

cell cholesterol homeostasis by upregulating cholesterol biosynthesis and redistributing cholesterol to viral replication membranes (Mackenzie et al. 2007); the same pattern has been shown in both Dengue virus (Heaton et al. 2010) and HIV (Taylor et al. 2011). It is thus quite likely that natural selection has favored host mechanisms that reduce or sequester cholesterol as a means of combating pathogens.

The conjunction of progesterone-driven risk of infection and cholesterol dependence in pathogens

In order to test our hypothesis that progesterone's effects on cholesterol constitute a second-order adaptation that reduces the costs of progesterone's immunomodulatory effects, we turn to an examination of the postulated selection pressures at issue. Specifically, if cholesterol reduction is a preemptive defensive maneuver aimed at decreasing the threat posed by those pathogens that stand to benefit from progesterone's immunomodulatory effects – most notably including those pathogens that pose a substantial risk to mother and conceptus – then it should be the case that a majority of such pathogens are importantly dependent on host cholesterol for their success. We therefore conducted an extensive literature search to identify such pathogens, then explored the extent to which they are known to be dependent on cholesterol. Table 1 presents our findings.

As evident in Table 1 and illustrated in Figure 1, a wide range of pathogens utilize cholesterol for maximal infectivity. As illustrated in Figure 2, almost all of these are best countered by a Th1 cytokine response in the host, and, as illustrated in Figure 3, a large number are exacerbated by a Th2 cytokine response. Progesterone shifts the Th1/Th2

balance toward the latter; therefore, it follows that progesterone increases the susceptibility of the host to the pathogens listed. As evident in Figure 4, many of these pathogens also pose a substantial risk during early pregnancy, a period characterized by a 'perfect storm' of minimal immunological capacities and maximal susceptibility to perturbation. Progesterone's general reduction of cholesterol, and the first-trimester nadir in maternal cholesterol in particular, thus appears to reflect a beneficial adaptation that helps protect both mother and conceptus from pathogenic infection.

Cholesterol during pregnancy

Despite progressive increases in progesterone levels, cholesterol levels increase during gestation; plasma concentration increases about 50% on average, the major increase occurring during the second trimester (Potter and Nestel 1979; Basaran 2009), while plasma triglyceride concentration reaches a peak in the third trimester (Potter and Nestel 1979; Basaran 2009). In regard to both LDLs and HDLs, the ratio of triglycerides to cholesterol rises throughout the course of pregnancy (Potter and Nestel 1979). At the proximate level, the increase in maternal cholesterol is likely due to the effects of estrogens, which elevate cholesterol significantly (Shchaefer et al. 1983). Estrogen increases progressively throughout pregnancy (Hassiakos et al. 1991). Levels of LDL parallel this rise, and the same is true of HDL through mid-pregnancy (maternal HDL levels fall late in pregnancy, possibly due to the onset of insulin resistance, glucose intolerance, and enhanced fatty acid mobilization – Ordovas et al. 1984). At the ultimate level, these increases can be correlated to the fetus's increased need for cholesterol. Fetal cholesterol is very high at the end of the second trimester, a period vital to the neural and vascular

440 growth of the developing organism (Herrera and Amusquivar 2000). Cholesterol
441 accessibility in the second and third trimesters helps enhance basic fetal metabolism and
442 function via normalized membrane integrity and cell signaling (Woollett 2005).
443 Cholesterol is used by the placenta for steroid synthesis, and fatty acids are used for
444 placental oxidation and membrane formation (Mankuta et al. 2010). The third trimester is
445 also marked by body fat accretion in the fetus, a process fundamentally dependent on
446 maternal cholesterol (Herrera and Amusquivar 2000).
447 Importantly, as pregnancy progresses, the fetus becomes increasingly buffered from
448 infection, and fetal development becomes increasingly less vulnerable to perturbation
449 (reviewed in Profet 1992). Meanwhile, the fetal demand for cholesterol continues to climb
450 throughout development, with the fetus matching any increase in maternal cholesterol
451 intake with corresponding elevations in fetal cholesterol uptake (Burke et al. 2009). As
452 reflected by the correlation between elevated maternal cholesterol and increased fetal
453 growth rates (McConihay et al. 2000), just as vulnerability to infection and susceptibility to
454 perturbation decline over the second and third trimesters, so too does the fetal need for
455 cholesterol increase. Against this backdrop, the steady increase in cholesterol across the
456 second and third trimesters is understandable as an adaptive pattern, reflecting a
457 reduction in the immunological costs of cholesterol and an increase in the need for this
458 vital building block.
459
460 **Conclusion**
461 Cholesterol modulation appears to be exquisitely timed over the course of
462 pregnancy, closely matching the shifting importance of combating pathogens and building

463 fetal tissue. The functionality of these changes is evident in the closeness of fit between
464 pathogens' ability to exploit progesterone-induced downregulation of inflammatory
465 responses and their reliance on cholesterol. Taken together, these features indicate that
466 the relationship between progesterone and cholesterol, though puzzling at first glance,
467 most likely reflects a second-order adaptation selected for by the increased vulnerability to
468 infection that is an inherent consequence of progesterone's role in maternal immune
469 tolerance of the conceptus. Indeed, it is possible that this is but one in a suite of second-
470 order adaptations serving this purpose. Progesterone may exercise a similar antagonistic
471 effect on the availability of iron (see Fessler 2002), and there is evidence that estrogen has
472 an antagonistic effect on the availability of tryptophan (Doyle et al. 2007). Like cholesterol,
473 iron and tryptophan play critical roles in infection, suggesting the presence of evolved
474 systems that compensate for the liabilities entailed by reproductive immunomodulation
475 (Fessler 2002; Doyle et al. 2007).

476 The approach presented here has a number of possible implications for both clinical
477 practice and basic research. First, if we are correct that cholesterol-dependent pathogens
478 pose a substantial risk to the conceptus, then, via pathways different from those recognized
479 to date in the literature (e.g., Bartels and O'Donoghue 2011), chronically high cholesterol
480 levels may constitute an under-recognized factor in both pregnancy loss and a variety of
481 developmental abnormalities. Second, if, as seems plausible, multiple feedback
482 mechanisms link progesterone production to cholesterol, then pharmacological
483 manipulation of cholesterol levels may entail unintended consequences for progesterone
484 production, with subsequent effects on fertility and other aspects of health. Third, in light
485 of existing evidence that progesterone shapes behavioral disease avoidance in a manner

that partially compensates for the immunomodulation effects of this hormone, if the thesis presented here is correct, this would constitute a case of two entirely independent compensatory mechanisms linked to a single proximate system. The latter suggests that evolutionary investigations of health and disease should attend carefully to the possibility of complex, and even multiple, second-order adaptations stemming from constraints on the optimality of individual adaptations.

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Table 1: Summary of survey of pathogens with regard to limiting and exacerbating cytokine responses, threat posed during pregnancy, and utilization of cholesterol.

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Pathogen	Limiting Cytokine Response	Exacerbating Cytokine Response	Threat during pregnancy	Cholesterol utility / dependence?
Toxoplasma gondii	Th1: IL-12, IL-6, TNF-alpha [1, 2]	Th2: IL-4 [1, 2]	Early pregnancy [3]	Yes [4]
Escherichia coli	Th1: IL-1, IL-2, IFN-gamma [5]	Th2: IL-10 [6]	Early pregnancy [7]	Yes [8]
Brucella abortus, Brucella melitensis	Th1: IL-12, IFN-gamma, TNF-alpha [9]	Th2: IL-10, IL-4 [10]	Early pregnancy [83]	Yes [11]
Shigella dysenteriae	Th1: IL-8, IL-2 [12]		Early pregnancy [64]	Yes [13]
Campylobacter jejuni	Th1: TNF-alpha, IL-8 [14]		Early pregnancy [15]	Yes [16]
Mycobacterium	Th1 [19]	Th2 [19]		Yes [17]
Coxsackievirus B	Th1: IFN-gamma [20]	Th2 [20]	Early pregnancy [54]	Yes [35]
Lassa virus	Th1: IL-6, IL-1beta [29]		Third trimester [53]	Yes [18]
Poliovirus	Th1: IL-6, IL-8 [21], IFN response [24]			Yes [23]
CVB4	Th1: IFN-gamma [25]			Yes [26]
Helicobacter pylori	Th1: IFN-gamma [27]	Th2: IL-4 [28]		Yes [36]
Leishmania donovani	Th1: IFN-gamma, IL-12 [30]	Th2: IL-4, IL-5, IL-10 [30]		Yes [31]
Plasmodium falciparum	Th1: IFN-gamma [32], TNF-alpha [33]			Yes [37]
Ehrlichia chaffeensis	Th1: IFN-gamma [34]			Yes [38]
Salmonella enterica	Th1: IFN-gamma, TNF-alpha [39]	Th2: IgG1/IgE [40], [41], Anti-Th1: decreases in IL-12, IFN-gamma cause increased and prolonged		Yes [42]

Epstein-Barr virus	Th1: IFN-gamma [46]	Th2 [47]		Yes [48]
Vesicular stomatitis virus	Th1 [49]			Yes [50]
Dengue virus	Th1: IFN-gamma [51]			Yes [52]
Varicella (Chickenpox)	Th1: IL-6 [57], IFN-gamma, IL-10, IL-12 [58]		Early pregnancy [59]	Yes [56]
Parvovirus	Th1: IL-10, IFN-gamma [60]		Early pregnancy [59]	Yes [61]
Rubella	Th1: IL-2, TNF-alpha [62]		Early pregnancy [59]	Yes. May depend on macropinocytosis for entry [69], which requires cholesterol [70]
Influenza virus	Th1: IL-6 [63]	Th2: IL-4 [64]	Early pregnancy [66]	Yes, decrease in infectivity when cholesterol depleted [65]
Seasonal Influenza A (H1N1)	Mixed response: TNF-alpha, IL-6, IL-8, IL-15 [87]		Early pregnancy [86]	
Adenoviruses	Th1: IL-6, IL-8, TNF [67]		Early pregnancy [72]	Yes [71]
Respiratory Syncytial Virus	Th1 [68]	Th2: IL-4 [73]		Yes [74]
Anaplasma phagocytophilum	Th1: IFN-gamma [77]	Th2 [76]		Yes [75]
Listeria monocytogenes	Th1: IL-12 [79]		Early pregnancy [80]	Yes [78]
Clostridium perfringens	Th1: IL-10[82]			Yes [81]
Leptospira	Th1: IL-12, TNF-alpha, IFN-gamma [85]			

Borna Virus	In acute infections, Th1: TNF-alpha, IL-2, IL-6, IFN-gamma [88] In chronic, switches to Th2 [88]	Yes [84]
cytomegalovirus		Can damage fetus at any stage in pregnancy [55]

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Figure 1: Fraction of pathogens examined that utilize cholesterol (N = 32).

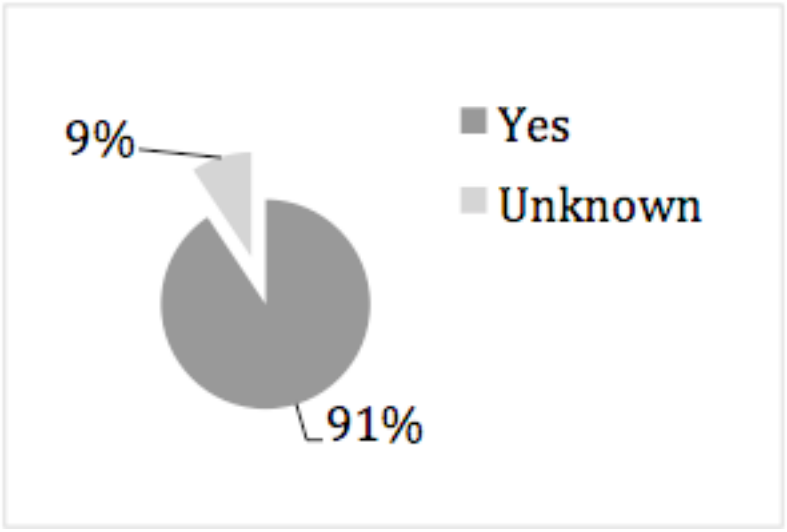

Figure 2: Pathogens for which the infection cycle is maximally limited by Th1 or Th2 responses (N = 32).

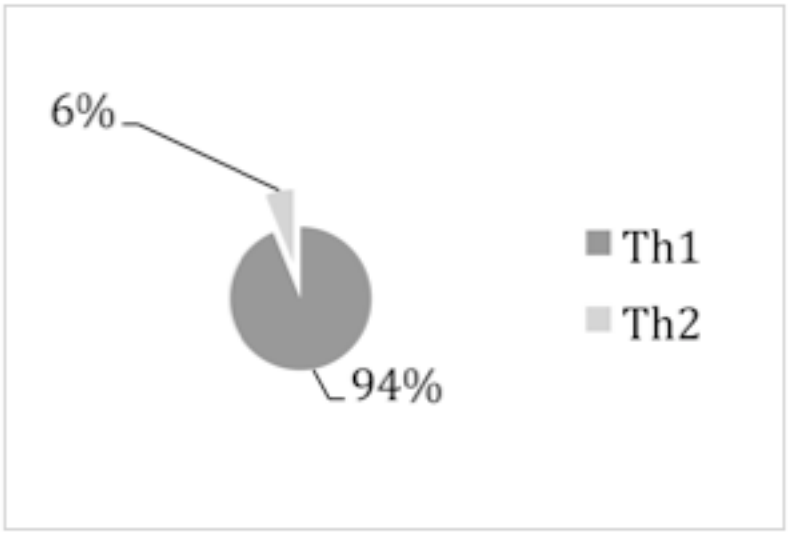

Figure 3: Pathogens for which the infection cycle is maximally enhanced by Th1 or Th2 responses (N = 15).

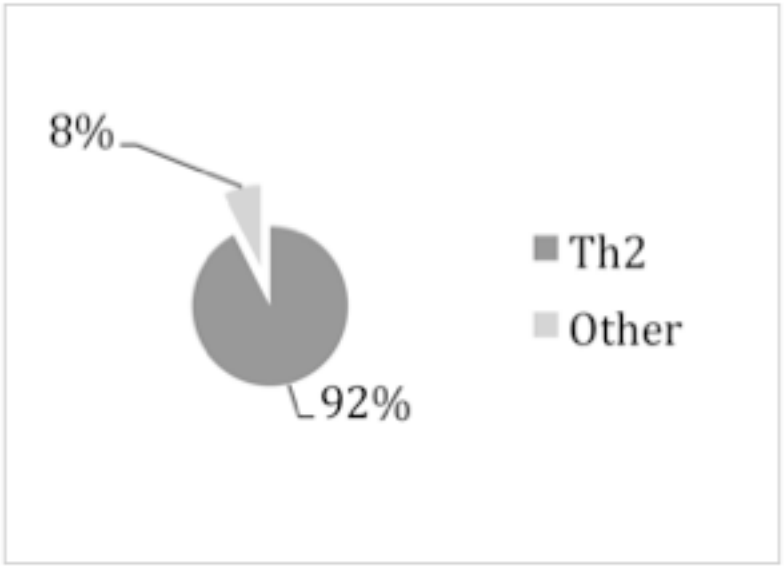

Figure 4: Period of maximal negative impact of infection among pathogens known to affect the course of pregnancy (N = 15).