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 triglycerides 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 (Woollett 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 (Safer 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 efflux 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 acetyltransferase (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
enhanced in anticipation of late pregnancy, when rapid fetal growth will require maternal
catabolism. In this view, the initial reduction in gestational cholesterol levels is simply a
side-effect of the need to lay in energy stores for later. However, the ratio of cholesterol to
triglycerides in fat cells is both constant and largely independent of cell size, indicating that
both are likely deposited simultaneously in a fixed ratio (Kovanen et al. 1975) – a feature
inconsistent with progesterone’s disruption of cholesterol production. Hence, while there
is conclusive evidence of a patterned shift from anabolism to catabolism across pregnancy,
this pattern provides neither proximate nor ultimate explanations of the antagonistic
effects of progesterone on cholesterol synthesis. Rather, we propose that the solution to
this puzzle lies in the intersection of the effects of progesterone on the immune system and
the role of cholesterol in infection.

**Progesterone-induced immunomodulation and compensatory prophylaxis**

With half of its genome being paternally derived, from the perspective of the
maternal immune system, the conceptus constitutes a genetically incompatible allograft.
As a consequence, changes must occur in the maternal immune system in order to prevent
maternal lymphocytes from attacking the conceptus (Szekeres-Bartho et al. 1983).
Pregnancy is facilitated by a shift in the Th1/Th2 balance in maternal immune functioning,
a move away from those inflammatory responses that pose the greatest danger to the
invasive blastocyst and the subsequently semi-parasitic embryo (reviewed in Fessler 2002;
Doyle et al. 2007; and Fleischman and Fessler 2011). Importantly, progesterone plays a
central role in the immunomodulation necessary to tolerate the half-foreign conceptus
(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).
Progesterone’s effects on the immune system clearly constitute an adaptation that serves to allow for gestation. Yet, this adaptation comes at the cost of increased susceptibility to infection during a particularly vulnerable period. Importantly, natural selection is not a teleological process – innovations that solve one problem can create another. Moreover, the liabilities entailed by one trait can, in turn, constitute a source of selective pressure leading to the evolution of new traits that mitigate the costs of these liabilities. Such second-order adaptations have been variously referred to as adaptive workarounds (Eastwick 2009) or the product of compensatory mutations (Maisnier-Patin and Andersson 2004). Of relevance to the matter at hand, recent evidence suggests that changes in other systems adaptively mitigate the vulnerability to pathogens entailed by progesterone’s effects on the immune system. Specifically, alterations in behavior provide one avenue for such mitigation. The compensatory prophylaxis hypothesis holds that, because prophylactic behavior entails time, energy, attention, and opportunity costs, rather than remaining constant, prophylactic behavior should be enhanced during periods of increased susceptibility to infection, when the greater benefits obtained merit increased expenditures (Fessler and Navarrete 2003). Hence, because progesterone regulates a cascade of physiological events that result in increased vulnerability to infection, progesterone can be expected to also mediate increased behavioral prophylaxis (Fessler and Navarrete 2003). Consonant with this hypothesis, studies have shown increases in disgust sensitivity, a proximate mechanism subserving disease avoidance, during the vulnerable first trimester (Fessler et al. 2005). More specifically, disgust sensitivity, disease-avoidance behaviors, and related perceptions and attitudes all increase as a function of progesterone levels (Fleischman and Fessler 2011; Navarete et al. 2007;
Conway et al. 2007; but see also Fessler and Navarrete 2003). Likewise, preferences for healthy over unhealthy faces (a cue of disease risk) are elevated during periods of elevated progesterone (Jones et al. 2005). Although compensatory prophylaxis is behavioral, we believe that a similar logic explains the effects of progesterone on cholesterol, as the latter plays a key role in infection.

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Critically, cholesterol plays a key role in infection. Lipid rafts are sites of entry and exit for a wide variety of viruses (Medigeshi et al. 2008). Lipid rafts can be exploited by pathogens in a number of ways. Some viruses, such as human immunodeficiency virus type 1, coxsackievirus, simian virus 40, and severe acute respiratory syndrome coronavirus, depend on lipid rafts for binding to and entry into the host cell; other viruses, such as rotavirus, Newcastle disease virus, influenza virus, Ebola virus, and Marburg virus, utilize raft-mediated pathways for assembly and egress (Chazal and Gerlier 2003; Manes et al. 2003; Ono and Freed 2005; Pelkmans 2005).

A number of bacteria similarly exhibit cholesterol dependence, including *Anaplasma phagocytophilium* (Xiong et al. 2009), *Escherichia coli* (Goluszko and Nowicki 2005), *Mycobacterium* (Gatfield and Pieters 2000), *Staphylococcus aureus* (Liu et al. 2008), *Salmonella* (Hayward et al. 2005), and *Shigella* (Hayward et al. 2005), among others. Some, such as *Mycobacterium tuberculosis*, utilize cholesterol as a primary carbon source throughout the course of infection, such that degradation of this sterol is crucial for bacterial persistence (Miner et al. 2009). In other cases, in species such as *Staphylococcus 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 pathogens.

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
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
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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
growth of the developing organism (Herrera and Amusquivar 2000). Cholesterol accessibility in the second and third trimesters helps enhance basic fetal metabolism and function via normalized membrane integrity and cell signaling (Woollett 2005). Cholesterol is used by the placenta for steroid synthesis, and fatty acids are used for placental oxidation and membrane formation (Mankuta et al. 2010). The third trimester is also marked by body fat accretion in the fetus, a process fundamentally dependent on maternal cholesterol (Herrera and Amusquivar 2000).

Importantly, as pregnancy progresses, the fetus becomes increasingly buffered from infection, and fetal development becomes increasingly less vulnerable to perturbation (reviewed in Profet 1992). Meanwhile, the fetal demand for cholesterol continues to climb throughout development, with the fetus matching any increase in maternal cholesterol intake with corresponding elevations in fetal cholesterol uptake (Burke et al. 2009). As reflected by the correlation between elevated maternal cholesterol and increased fetal growth rates (McConihay et al. 2000), just as vulnerability to infection and susceptibility to perturbation decline over the second and third trimesters, so too does the fetal need for cholesterol increase. Against this backdrop, the steady increase in cholesterol across the second and third trimesters is understandable as an adaptive pattern, reflecting a reduction in the immunological costs of cholesterol and an increase in the need for this vital building block.

**Conclusion**

Cholesterol modulation appears to be exquisitely timed over the course of pregnancy, closely matching the shifting importance of combating pathogens and building
fetal tissue. The functionality of these changes is evident in the closeness of fit between pathogens’ ability to exploit progesterone-induced downregulation of inflammatory responses and their reliance on cholesterol. Taken together, these features indicate that the relationship between progesterone and cholesterol, though puzzling at first glance, most likely reflects a second-order adaptation selected for by the increased vulnerability to infection that is an inherent consequence of progesterone’s role in maternal immune tolerance of the conceptus. Indeed, it is possible that this is but one in a suite of second-order adaptations serving this purpose. Progesterone may exercise a similar antagonistic effect on the availability of iron (see Fessler 2002), and there is evidence that estrogen has an antagonistic effect on the availability of tryptophan (Doyle et al. 2007). Like cholesterol, iron and tryptophan play critical roles in infection, suggesting the presence of evolved systems that compensate for the liabilities entailed by reproductive immunomodulation (Fessler 2002; Doyle et al. 2007).

The approach presented here has a number of possible implications for both clinical practice and basic research. First, if we are correct that cholesterol-dependent pathogens pose a substantial risk to the conceptus, then, via pathways different from those recognized to date in the literature (e.g., Bartels and O’Donoghue 2011), chronically high cholesterol levels may constitute an under-recognized factor in both pregnancy loss and a variety of developmental abnormalities. Second, if, as seems plausible, multiple feedback mechanisms link progesterone production to cholesterol, then pharmacological manipulation of cholesterol levels may entail unintended consequences for progesterone production, with subsequent effects on fertility and other aspects of health. Third, in light 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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the murine model of human anaplasmosis. *FEMS Immunology & Medical Microbiology* 50(3):401-410.


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

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Limiting Cytokine Response</th>
<th>Exacerbating Cytokine Response</th>
<th>Threat during pregnancy</th>
<th>Cholesterol utility / dependence?</th>
</tr>
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<tbody>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>Th1: IL-12, IL-6, TNF-alpha [1, 2]</td>
<td>Th2: IL-4 [1, 2]</td>
<td>Early pregnancy [3]</td>
<td>Yes [4]</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>Th1: IL-8, IL-2 [12]</td>
<td></td>
<td>Early pregnancy [64]</td>
<td>Yes [13]</td>
</tr>
<tr>
<td><em>Lassa virus</em></td>
<td>Th1: IL-6, IL-1beta [29]</td>
<td></td>
<td>Third trimester [53]</td>
<td>Yes [18]</td>
</tr>
<tr>
<td><em>Poliovirus</em></td>
<td>Th1: IL-6, IL-8 [21], IFN response [24]</td>
<td>Th2: IL-4 [23]</td>
<td></td>
<td>Yes [23]</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>Th1: IFN-gamma [27]</td>
<td>Th2: IL-4, IL-5, IL-10</td>
<td></td>
<td>Yes [36]</td>
</tr>
<tr>
<td><em>Leishmania donovani</em></td>
<td>Th1: IFN-gamma, IL-12 [30]</td>
<td>Th2: IL-4, IL-12, IL-10 [30]</td>
<td></td>
<td>Yes [31]</td>
</tr>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td>Th1: IFN-gamma [32], TNF-alpha [33]</td>
<td>Th2: IL-4, IL-5, IL-10</td>
<td></td>
<td>Yes [37]</td>
</tr>
<tr>
<td><em>Ehrlichia chaffeensis</em></td>
<td>Th1: IFN-gamma [34]</td>
<td>Th2: IgG1/IgE [40], [41], Anti-Th1: decreases in IL-12, IFN-gamma cause increased and prolonged</td>
<td></td>
<td>Yes [38]</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>Th1: IFN-gamma, TNF-alpha [39]</td>
<td>Th2: IgG1/IgE [40], [41], Anti-Th1: decreases in IL-12, IFN-gamma cause increased and prolonged</td>
<td></td>
<td>Yes [42]</td>
</tr>
<tr>
<td>Virus Type</td>
<td>Th1</td>
<td>Th2</td>
<td>Early Pregnancy</td>
<td>Yes or No</td>
</tr>
<tr>
<td>------------</td>
<td>-----</td>
<td>-----</td>
<td>-----------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Vesicular stomatitis virus</td>
<td>Th1 [49]</td>
<td></td>
<td>Yes [50]</td>
<td></td>
</tr>
<tr>
<td>Dengue virus</td>
<td>Th1: IFN-gamma [51]</td>
<td></td>
<td>Yes [52]</td>
<td></td>
</tr>
<tr>
<td>Varicella (Chickenpox)</td>
<td>Th1: IL-6 [57], IFN-gamma, IL-10, IL-12 [58]</td>
<td>Early pregnancy [59]</td>
<td>Yes [56]</td>
<td></td>
</tr>
<tr>
<td>Parvovirus</td>
<td>Th1: IL-10, IFN-gamma [60]</td>
<td>Early pregnancy [59]</td>
<td>Yes [61]</td>
<td></td>
</tr>
<tr>
<td>Rubella</td>
<td>Th1: IL-2, TNF-alpha [62]</td>
<td>Early pregnancy [59]</td>
<td>Yes, may depend on macropinocytosis for entry [69], which requires cholesterol [70]</td>
<td></td>
</tr>
<tr>
<td>Influenza virus</td>
<td>Th1: IL-6 [63]</td>
<td>Th2: IL-4 [64]</td>
<td>Early pregnancy [66]</td>
<td></td>
</tr>
<tr>
<td>Seasonal Influenza A (H1N1)</td>
<td>Mixed response: TNF-alpha, IL-6, IL-8, IL-15 [87]</td>
<td>Early pregnancy [86]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>Th1: IL-6, IL-8, TNF [67]</td>
<td>Early pregnancy [72]</td>
<td>Yes [71]</td>
<td></td>
</tr>
<tr>
<td>Respiratory Syncytial Virus</td>
<td>Th1 [68]</td>
<td>Th2: IL-4 [73]</td>
<td>Yes [74]</td>
<td></td>
</tr>
<tr>
<td>Anaplasma phagocytophilum</td>
<td>Th1: IFN-gamma [77]</td>
<td>Th2 [76]</td>
<td>Yes [75]</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>Th1: IL-12 [79]</td>
<td>Early pregnancy [80]</td>
<td>Yes [78]</td>
<td></td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>Th1: IL-10 [82]</td>
<td></td>
<td>Yes [81]</td>
<td></td>
</tr>
<tr>
<td>Leptospira</td>
<td>Th1: IL-12, TNF-alpha, IFN-gamma [85]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table lists various viruses and their associated immune response markers. For some viruses, the response includes specific cytokines such as IFN-gamma, TNF-alpha, and IL-10. Early pregnancy responses are indicated for several viruses, and some viruses have additional notes regarding potential dependency on cholesterol and macropinocytosis for entry.
<table>
<thead>
<tr>
<th>Virus</th>
<th>In acute infections, Th1: TNF-alpha, IL-2, IL-6, IFN-gamma [88]</th>
<th>In chronic, switches to Th2 [88]</th>
<th>Yes [84]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borna Virus</td>
<td></td>
<td></td>
<td>Can damage fetus at any stage in pregnancy [55]</td>
</tr>
</tbody>
</table>
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).