Lactation from the inside out: Maternal homeorhetic gastrointestinal adaptations regulating energy and nutrient flow into milk production

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	Lactation invokes homeorhetic processes to ramp up and supply milk synthesis components to fulfil nutritional, immunological and microbiological requirements of developing offspring, overseen by complex neuroendocrine networks. The maternal gut meets these intense metabolic demands, supported by hyperphagia and rapid adjustments to process larger food quantities. Enteroplasticity describes an inherent ability of the gastrointestinal tract to harness metabolic and structural adaptations that increase nutrient absorption. Most shifts in response to increased demands are transitory and by secreting milk, the continuous energetic drain out of the maternal body avoids development of pathological metabolic diseases. Lactation has various positive benefits for long-term maternal health but many females do not lactate for long post pregnancy and younger women are increasingly pre-disposed to excessive body mass and/or metabolic complications prior to reproducing. Inadvertently invoking intestinal adaptations to harvest and store excess nutrients has negative health implications with increased risks for both mother and offspring.

Focus of review

This review focuses on the concept of enteroplasticity – digestive flexibility or gut remodelling – adaptations that are potentially available or deployed during lactation, looking more closely where possible at the different forms, but also timing, of gastrointestinal changes that take place to increase physical capacity or the metabolic means to harvest and absorb more nutrients under conditions of high energetic demand. Where there are associations with maternal health, the relationships between gut and health/dysbiosis or metabolic perturbations are considered, particularly whether any of these changes remain after lactation ends. What limited evidence that is available, comes from disparate fields and sources, due to the very restricted opportunities to study the human maternal gut, thus the caveat is, that whilst animal studies in many different situations can offer intriguing insights, they may not apply in the same way, or to an equivalent extent, in the human form.

1. Introduction

For many female animals, reproduction involves the most energetically costly life stages. A multitude of changes need to take place as she quickly responds and adapts to new conditions necessary to produce offspring on top of normal functioning. Lactation is a natural physiological state and an extension of the reproductive process for some. It is a defining feature of mammals as they continue to provide complete nutrition to support their developing offspring until they are fully weaned onto externally available food sources. Endocrine lactogenic hormone changes associated with birth stimulate the onset of lactation, following earlier mammary gland growth and differentiation during gestation. The new suckling stimulus initiates profound changes in brain function via the hypothalamus (Smith and Grove, 2002) although other unseen internal preparations will also have taken place throughout the gravid body. Metabolic changes, increased blood flow and expanded adipose lipid reserves are all preparations needed for the new energetic demands of milk production. The onset of lactation marks the end of a pregnancy but the transition to an even higher metabolic load. The early stages often lead to a temporary state of whole-body negative energy balance, as inputs cannot initially keep up with the high priority outputs. The extent of negative energy balance experienced is variable but can be prolonged (weeks/months). Another common feature can be a temporary cessation of ovarian cyclicity and

reproductive function which reflects how profound these energetic impacts can be, but also makes sound evolutionary survival sense. in addition to providing a temporary contraceptive effect, a non-fertile period provides spacing between offspring to allow for maternal recovery (Robyn et al., 1986). Despite these seemingly disruptive effects, an uncomplicated lactation does provide a range of positive benefits for maternal physical health and well-being (Table 1, adapted from Del Ciampo and Del Ciampo, 2018 and other sources) although further research is needed to unravel many of the unresolved complexities.

The dam's ability to meet metabolic demands and supply adequate nutrition to developing offspring is determined by external availability coupled with internal capacity and provision, with appropriate timing. Where the stimulus for milk secretion occurs within the norms for a particular species of their physiological capacity and limits, this relates closely to the expected number of offspring that can be easily supported, which is often mirrored by the number of mammary gland nipples available. Much emphasis is often placed on the benefits of lactation for offspring (Geddes et al., 2021), less so on any direct benefits for the mother (reviewed by Del Ciampo and Del Ciampo, 2018; Table 1) or conversely the health impact and risks of how what takes place during the ensuing lactation period(s) may additionally impact maternal ongoing health (Farpour-Lambert et al., 2018). An impaired gestation and lactation process or insufficient maternal adaptations have the potential to disrupt the supply of nutrients to the fetus or milk to the neonate, affecting their immediate health (Lunn, 1994; Kuhn et al., 2005; Del Ciampo and Del Ciampo, 2018), or additionally have generational effects via developmental programming. For modern humans, some are still subject to varying degrees of inadequate nutrition and increasing numbers are exposed to over-nutrition and suboptimal diets during pregnancy and lactation, both of which could impact the dyad. Therefore these are timely intervention points to get important family health impact messages across to new parents.

Dairy cows provide opportunities to explore a model of extreme lactation. Substantial amounts of research have taken place in production animals and a great deal of knowledge gained about metabolism of lactation in ruminants from commercial milk farms (Baumgard et al., 2017). The calf is removed from its dam following birth and artificially fed if retained within the production system. Milk is removed from the dam's mammary glands by artificial means, usually mechanically expressed, thereby uncoupling natural demand and providing maximal continuous milk synthesis stimulation. Moreover, many commercial dairy cows have been artificially selected for high endogenous lactogenic growth hormone concentrations and bred for increased milk quality or production traits to further maximise milk yields. Dairy interests are directed towards maximising lifetime output and much emphasis is placed on what inputs can achieve this, which led to the apt description that the dairy cow is an appendage to her mammary gland (Baumgard et al., 2017). High yielders in particular often have an extreme negative energy imbalance in peak lactation and there are distinct metabolic diseases of lactation that reflect such disturbances (p984, Sundrum, 2015). Dairy cows can also have problems with re-establishment of normal ovarian cyclicity post partum, beyond any simple contraceptive effect, with disrupted fertility (Taylor et al., 2004). Whereas sheep, another ruminant, are established models of gestational health outcomes, with research into effects of poor nutrition during pregnancy via the placental unit on offspring development. Such studies have provided essential insights, especially as similar observations can only be made indirectly in humans.

Although a lack of detailed information covering the norm of human lactation from the inside out persists, Geddes et al. (2021) have reviewed the current state-of-play using a biological systems approach. Much of this paucity of knowledge about human lactation remains as it cannot be obtained non-invasively, and ethically, it is unacceptable to experimentally investigate human pregnancy and lactation where there could exist any possible risks to the development of the baby or health of both. The situation is compounded as there is an unbalanced scientific knowledge base, as the majority of general biology and clinical research has taken place predominantly using male subjects (Beery and Zucker,

Table 1
Potential positive health benefits for human mothers based on lactation duration (adapted from Del Ciampo and Del Ciampo, 2018; Hartmann et al., 1998; Oboh et al., 2021).

Immediate post partum effects:	Risk factor	Long term effects		Risk factor
		Reduced risks for:		
Reduced adiposity and body mass	Increased OXT – decreased insulin-resistance	Decreased BMI 6-12 months post partum for exclusive breastfeeding	**Excess body mass** Reduced adiposity and body mass with lower BMI	Further benefits of breastfeeding >10 months duration
			Diabetes Metabolic syndrome Cardiovascular diseases OXT - decreased insulin-resistance	12% decrease/year
Uterine involution	Increased OXT	Reduced infection and haemorrhage	**Endometrium cancer Endometriosis**	8% decrease, greater with cumulative lactation time
Lactation-induced amenorrhea	Increased PRL inhibiting P_4, E_2 and ovulation	Reduced (immediate) chance of pregnancy	**Ovarian cancer** Decreased LH, E_2 protective	2% decrease/year
Reduced stress, anxiety and depression	Daytime cortisol stable; OXT, PRL anxiolytic. (Hartmann et al., 1998)		**Breast cancer** Decreased E_2, decreased cell proliferation and differentiation following mammary gland involution (increased apoptosis)	4% decrease/year
			Osteoporosis Compensatory mechanisms can increase intestinal and renal absorption, also calcium mobilisation (dependent on Vitamin D status). Recovery of bone mineral density depends on lactation duration or time of weaning.	Potentially reduced
			Multiparity	Protective effect studies needed (Oboh et al., 2021)
			Alzheimer's disease E_2 protective	
			Multiple sclerosis E_2 protective	Linear decrease with duration

E_2 – estradiol; LH – luteinising hormone; OXT – oxytocin; P_4 – progesterone; PRL – prolactin.

2011), with the obvious exception of lactation, plus pregnancy and aspects of reproduction that can only be studied in females. This issue has inadvertently been perpetuated whilst trying to avoid the additional complexity of different stages of the ovarian hormone cycle, with supposedly higher response variability (Cavalcanti-de-Albuquerque and Donata, 2021), but such a limited view is a scientific practice issue that needs to be resolved with improved experimental design and statistical analyses. It is now acknowledged, with redress being instigated by some research funding bodies, journal publishers, pharmaceutical companies and to fulfil societal expectations.

Natural selection acts differently on mating and reproductive traits leading to biological sex differences. The dissimilarities between females and males in fundamental aspects of metabolic homeostasis and the recruitment of further homeorhetic responses to short-term challenges remain important to distinguish as they mediate different responses on physical outcomes. These include body mass composition and control and therefore susceptibilities to associated metabolic disturbances, comorbidities and in particular, reaction to pharmaceuticals and surgical procedures, notably bariatric procedures. Even though lactation is a temporary physiological state unique to biological females, uncovering how its response mechanisms operate could be more widely beneficial, providing general and specific insights into whole-body responses to energetic challenges, especially body mass control (Farpour-Lambert et al., 2018) but additionally for physical activities including athletic performance and recovery; all with important long-term health implications.

Whilst this review focuses on the immediate gastrointestinal changes occurring during a single lactation period, exploring mammalian abilities to respond to dynamic changes, these cannot always, nor should be, separated from the preceding pregnancy. Some gestational adjustments may already have occurred, such as increased circulating blood volume and vascularity, gut, hormone and metabolic adaptations and some of these enhancements or effects may still persist postnatally or even be essential for lactation. Conversely, as global breastfeeding rates only average 41% (Geddes et al., 2021), an additional consideration for the majority of human mothers, who for medical or practical reasons, do not lactate or breast feed after having their infant, is that they may have retained some pregnancy-induced adaptations. Thus there are likely to be different metabolic and health implications for females that do not lactate but whose bodies, in preparation for lactation, have enlarged adipose lipid stores with metabolic priming and an enhanced ability to harvest more nutrients. The impact of not breast feeding on maternal health may necessitate additional postnatal guidance to improve the immediate metabolic status of these mothers and reduce their future disease risk factors.

2. Mammary glands – what demands do they place on the maternal body?

Mature mammary glands undergo repeated cycles of lobuloalveolar differentiation, milk synthesis and secretion in response to suckling (Capuco and Akers, 2009; Canul-Medina and Fernandez-Mejia, 2019). Mammary tissue has the unique ability to form lactose in large quantities from the simple sugars glucose and galactose using lactose synthase which has high affinity for glucose. Human milk contains many bioactive components, including milk oligosaccharides that can promote the growth of beneficial microbes, especially *Bifidobacteria,* although their profiles vary substantially amongst women, depending on their diet. With the production of ~800 ml/day of milk a woman can transfer about 200 mg calcium daily (Del Ciampo and Del Ciampo, 2018) and as well as providing nutrients, supplies immune cells associated with defence against bacterial infections. Immunoglobulins and antimicrobial peptides enhance mucosal barrier function in infant guts whilst promoting intestinal colonisation with appropriate *Lactobacilli* and other key components of a healthy microbiome (Rodríguez et al., 2021; Geddes et al., 2021). Once lactation is established, it is sustained by

systemic hormones (variable by species, mainly PRL in rats, humans; GH/somatropin in ruminants; Hartmann et al., 1998), local factors, regular suckling stimulus and adequate water, nutrient and mineral delivery.

Multiple control systems are involved in the partitioning of available nutrients into normal maternal functioning and additionally into milk production. These involve major coordinated changes in maternal tissues, with shifts in mammary, liver and adipose tissue metabolism, throughout the different stages of lactation. The gut is a complex neuroendocrine organ that maintains a constant exchange of information with the brain via the gut-brain axis and enteric nervous system that has neurotransmitters and neural integration and processing that mirrors those in brain. The gut-brain axis has roles in maintaining whole-body energy balance via hypothalamic circuits that regulate feed intake/behaviour and activity/exercise. Chen et al. (1999) and Suzuki et al. (2013) reported mRNA levels of orexigenic agouti-related protein (AgRP) were significantly increased in the arcuate nuclei of lactating rats and furthermore (Suzuki et al., 2013) found that anorexigenic pro-opiomelanocortin (POMC) significantly decreased, suggesting that central mechanisms also shift to facilitate hyperphagia during lactation. Involvement of the melanocortin system implies nutrient, hormonal and neuronal inputs and involves modulation of functional connections of the gut with other organs, some more distant, through an integrated network of nervous, metabolic and endocrine signals and interconnected axes, reviewed by many others: gut-breast axis (Rodríguez et al., 2021), gut-pancreas axis (Polakof et al., 2011; GLP-1: Davis and Sandoval, 2020), reproduction and energy balance (Smith and Grove, 2002), immunity-metabolism cross-talk (Man et al., 2017).

The physiology of pregnancy is characterised by glucose metabolism shifts, with alterations in insulin sensitivity and hormone concentrations. Central nervous system, hypothalamic or peripheral resistance to hormones with a 'blunting' of signals e.g. high circulating prolactin and hypothalamic resistance to leptin allows uncoupling of normal restraints to become permissive for energy accumulation, leading to ~3 kg lipid storage accumulation in pregnant women. Thus preparatory changes take place during pregnancy prior to and anticipatory of an ensuing lactation, even though pregnancy itself is a time of high energy demands. Whereas pregnancy is characterised by notably altered cardiovascular capacity with a state of systemic vasodilation providing increased blood flow, most mammals revert during early lactation (2-5 weeks post partum) back to non-pregnant cardiovascular function as uterine involution takes place (Vonnahme and Lemley, 2011). Later lactation is more characterised by hyperphagia, but during early lactation, before any more extensive gut adaptations might occur, lipid stores are mobilised from depots generated during pregnancy that are more metabolically active. This has been measured as an average monthly reduction of 450 g in breastfeeding women (Del Ciampo and Del Ciampo, 2018).

2.1. Tipping the balance from homeostasis towards homeorhesis of pregnancy and lactation

Homeostasis maintains normal physiology and steady-state functioning whereas pregnancy and then lactation both initiate a coordinated ramping up and/or dampening of multiple systems towards a temporary new trajectory. These changes are more appropriately described as homeorhetic adjustments (Bauman et al., 1982; Sundrum, 2015) as they involve discrete time-dependent changes to regulatory processes thereby establishing a new heightened physiological state to specifically serve the highest priority functions of reproduction/survival that take precedence for partitioning of nutrients (Baumgard et al., 2017). They are characterised by chronic regulation (hours/days) instead of the acute regulation of homeostasis (seconds or minutes), although are still mediated through altered responses to homeostatic signals (Bell, 1995; Baumgard et al., 2017) and with simultaneous influence on multiple tissues (Hartmann et al., 1998).

For a relatively short but intense time period, and within a limited physical space, the maternal gastrointestinal tract must elevate its metabolic actions to deliver additional nutrients to the energy-demanding gravid uterus via the placenta. Homeorhetic nutrient partitioning shifts in liver (with increased hepatic gluconeogenesis from endogenous substrates), decreased peripheral tissue glucose utilisation, increased fatty acid mobilisation from adipose tissue (Chaves and Herrera, 1978) and increased amino acid mobilisation from muscle, with the gradual adoption of an insulin-resistant state during pregnancy; all work together to ensure both growth of the conceptus and development of mammary gland tissues at the same time as building up adipose lipid reserves (Martin-Hidalgo et al., 1994; Bell, 1995).

Following the onset of lactation, maternal tissues undergo more homeorhetic adaptations to further shift maternal metabolism to support milk synthesis and secretion in response to offspring suckling demands until the maturing young can process external forms of adequate nutrition. What causes these bigger shifts? For some species, timing of breeding is hard wired into circannual daylight cycles entrained by external photoperiod cues but other cues can be increased food intake or meal frequency which trigger organisms to anticipate and quickly prepare for precise changes (Hastings et al., 2007). Changes in molecular clocks during the transition from pregnancy to lactation in rats documented by Casey et al. (2009) in gene transcriptional profiles of mammary, liver and adipose tissues suggested that homeorhetic adaptation to lactation was mediated in part by the circadian system (Patel et al., 2011). They proposed that the central clock in the suprachiasmatic nuclei integrates received cues and responds to coordinate behavioural and physiological changes across the dam. Signalling to multiple peripheral tissues including endocrine glands, stimulated changes in tissue core molecular clocks, particularly mammary glands and liver – altering their metabolism by effectively 'turning' the proteome and metabolome of the dam to support lactation.

Lactation, with the preceding pregnancy, is thus made possible with extensive internal body changes, involving all body systems and most organs. What influences how extreme or prolonged these shifts need to remain in place is determined by the whole body energy balance - achieved when energy expenditure matches energy intake but considered negative when expenditure is higher than intake. The idea of balance is within a range of normal capacity to deal with some movement in both directions with minor adjustments, whereas too little or too much can both be disruptive to normal fertility functioning. In relation to dairy cows, Bell (1995) suggested that as the interrelations between body condition score, feed intake, milk production and health vary widely among individuals, this may be due to individual differences in capabilities to homeorheticly regulate nutrient partitioning (Hartmann et al., 1998). Various studies have also demonstrated flexibility in both homeostatic and homeorhetic adaptations to the energy demands for milk synthesis in women (Butte, 1998; Hartmann et al., 1998). This flexibility suggests that recommendations for dietary energy intake during lactation should address current body mass status and would address health implications of women more appropriately if they were based on the individual goals for postweaning body mass and personalised future health risks rather than the standardised energy requirements for milk production (Hartmann et al., 1995).

2.2. How the gut naturally responds to changing energy needs

Large but compact, and mostly unseen, the extensive and complex gastrointestinal system has multiple dynamic active and responsive tissues and organs, different cells, with the abundance, location and distribution of many only just being revealed (Burclaff et al., 2022). The notion that gut size increases can occur in response to hyperphagia during reproductive states is not particularly revealing in itself as lactation-induced gut lengthening has been noted in multi-offspring rodents since at least the 1960s and furthermore it is well known that specific areas of the gastrointestinal tract can undergo substantial

compensatory changes in concert with whole body responses to other developmental, reproductive changes and environmental challenges.

Enteroplasticity of the existing gastrointestinal tract is an inherent ability used by both sexes during development – such as the transition for offspring at weaning with the shift from high quality liquid milk easily digested by enzymes in the small intestine to solid mixed food matter with reduced digestibility and a later requirement for some microbial fermentation. Gut modifications, allowing digestive flexibility, have been observed in numerous natural and experimental settings (e.g. small rodent models with exercise or cold-induced adaptations), each situation providing different insights into very complicated processes. It is valid to ask whether the features of flexibility and capacity demonstrated from captive studies apply more widely, and a range of studies have confirmed enteroplastic changes in wild populations. When subject to reduced dietary quality, leaf-eating primates had enlarged caecums and the largest colon gut lengths (Chivers and Hladik, 1980). Conversely, temporary adjustments minimising organ/tissue masses have been documented in migratory birds (equivalent to in-flight starvation) and were consistently ranked for extent of phenotypic flexibility with; highest to lowest mass changes in: small intestine (40%), liver, kidney, gizzard, heart, flight and leg muscle (<20%). These consistent findings are suggestive of an underlying common mechanism (Bauchinger and McWilliams, 2010; McWilliams and Karasov, 2014) and the organ differences were directly related to tissue-specific protein turnover.

Living organisms require continuous replacement of energy sources that are in constant use for the essential functions of maintenance (survival), growth, and when conditions are appropriate or allow, require even more for reproduction. Lactation for mammals permits numerous evolutionary-honed biological advantages for the offspring but the trade-off is that it is the most energetically expensive time period for a female as she produces and exports quantities of energy-rich milk to growing neonates and/or infants. During pregnancy, the placenta is a temporary organ that provides an additional interface between maternal blood supplying the fetus with nutrients and gaseous exchange. During both pregnancy and lactation, the entire gastrointestinal tract, assisted by associated metabolically-active organs, including liver and pancreas, provide the permanent interface between available external nutrition/energy sources intake and uptake and is therefore, unsurprisingly, one of the most energetically expensive tissues to maintain (Naya et al., 2008). The existing system has inherent abilities to morphologically and functionally adapt to internal/external changing conditions and studies in rats indicate that the majority of the metabolic rate enhancement is due to increased mass of these organs with higher maintenance costs (Naya et al., 2008 citing Canas et al., 1982) thus there exists an additional energetic requirement to service expanded gut machinery tasked with dealing with increased amounts of food firstly during pregnancy but more so during lactation.

How does the body energetically finance this? It seems that even under energy restriction, the gut still retains some inherent adaptive potential which helps to explain conflicting or apparently paradoxical findings that are evident in different studies of caloric restriction (CR) with no or opposite effects on cell characteristics and small intestine mass changes. Peña-Villalobos et al. (2019) explored this further by analysing intestinal epithelium response (balance between cell proliferation and death) in rodents subjected for 20 days to treatments that varied in periodicity of feeding and dietary caloric content. They found that male mice adapted to a short-term 60% CR regimen with longer intestines and demonstrated caloric restriction induction of cell proliferation that synchronised the proliferation rate of stem and/or progenitor cells in the small intestinal crypts with mTORC1 as a key mediator resulting in increased crypt branching and intestinal tissue expansion with a growth rate of 0.22 cm/day. These changes together increased the digestive capacity of the mice as they had concomitant significantly lower energy content in their faeces. The authors have suggested that this rapid intestine plasticity could be explained by

mechanisms whereby cell proliferation and tissue turnover were balanced to yield positive consequences on metabolic performance. This provides a short-term mechanism that increases an animal's fitness when it is energy limited, even though this involved assigning additional maintenance energy costs and structural biomolecules to the expansion of the gut compartment responsible for the processing and absorption of nutrients, despite reductions in quantity and/or quality of available food.

2.3. Gut structure, functions and malfunctions

The gut is often considered as a single entire entity, its main feature being a large holding capacity for processing and transformation of ingested nutrients as they move down the tract, facilitated on the way by different functional units. As a whole, it undertakes essential roles in digestion, nutrient acquisition, transfer and absorption, maintaining ion balance, with production and secretion of hormones, neural (enteric nervous system) and other signalling molecules regulating eating behaviour and gut motility, plus nutrient sensors and hormone receptors for integration with regulation of whole-body energy homeostasis (Furness et al., 2013). It additionally has important immune functions, providing protection from parasites at the same time as housing and feeding the host gut microbiota, and last but not least, excretes the unutilised remnants of ingestion. The entire length of the gut is lined with a single-cell thick layer of epithelium separating the inside of the lumen from its contents (including various nutrients, microbes, parasites, toxins and drugs). There are many proliferative cells as the gut lining is constantly renewing as well as producing a protective layer of mucus. The average total mucosal surface of the human digestive tract is impressive at ~ 32 m^2 (Helander and Fändriks, 2014). Despite having many overlapping roles, the whole gastrointestinal system can be further subdivided into distinct functional compartments throughout the upper and lower digestive tracts. These have more defined areas of specialised tissues (Barker et al., 2010) with variable amounts of differentiated and stem cell types, including mucosal, serosal, immune and enteroendocrine cells.

The stomach receives food first and is responsible for the mechanical and digestive enzymatic processing of nutrients, whilst participating in the regulation of acid secretion, nutrient assimilation, hormone secretion contributing towards appetite control (e.g. orexigenic ghrelin), plus vagal signalling to the central nervous system that maintains whole body energy homeostasis. After exiting the stomach, contents progress into the larger upper compartment - small intestine - and then lower compartment - large intestine. Despite being continuous, these are spatially distinct organs with defined physiological functions that signal between (Wellman et al., 2022) and work together to maintain processes that contribute towards healthy functioning. The normal functions of the small intestine compartments (duodenum, jejunum, ileum) include nutrient acquisition by absorptive enterocytes, utilisation and maintaining immunocompetence. The different areas vary in biomechanical properties and the transition from ileum to colon is a segment containing Paneth cells, providing some protection against microorganisms, therefore without notable microbial fermentation. The colon has ascending, transcending, descending and sigmoid segments plus rodents additionally have a caecum or large sac-like organ important for bacterial fermentation and absorption. As it is extremely difficult to obtain insights into human gut functioning during reproductive states, findings from animal studies and human surgical interventions have to be relied upon to inform and progress our understanding about both normal and adaptive enteroplastic changes.

2.4. Loss of and alterations in gut function

The importance of the stomach to whole-body energy homeostasis beyond being the first holding vessel becomes obvious when faced with the wide-reaching and irreversible disruptive effects of total gastrectomy. Stomach removal leads to morphological and functional changes (faster emptying rate, diminished secretory capacity, nutrient form changes in lower areas) in the small intestine areas below i.e. the modified action of the mucosa, enteric neurons, enteroendocrine cells, gut peptides and altered intestinal barrier, including reduced expression of tight junction proteins (Puzio et al., 2021). The resultant loss of digestive enzymes and hormones affects appetite, causing metabolic complications, nutrient deficiencies (iron, vitamins); but also mechanical, circulatory problems and disrupted neural connections via the vagal nerve. Various studies indicate that the mechanisms responsible for post-operative complications involve independent alterations in vagal innervation, hormone signalling pathways, bile acids, gastrointestinal motility and intestinal microbiota (reviewed by Puzio et al., 2021). Whereas it can be presumed that increased mass of stomach tissue during pregnancy and lactation will lead to alternations and/or enhanced performance of many of these functions.

2.5. Compensatory gut growth

Another major cause of intestinal failure is short bowel (=small intestine) syndrome. Some natural compensatory processes occur after bowel resection in the remaining intestine to improve nutrient status, the extent of what is possible depends on the underlying medical condition and gut location involved (Neelis et al., 2016). Gut growth is clinically encouraged post-operatively with intestinally-derived growth factors such as glucagon-like peptide 2 (GLP-2) and enteral nutrition to stimulate adaptation in the remaining tissues to enhance this natural hyperplasia response, as the aim is to wean patients off parenteral nutrition. The continuous administration of nutrients is hypothesised to enhance absorption by maximising saturation of the carrier proteins thereby increasing intestinal function of remaining tissues.

Adaptations that have been observed in animal models of compensatory hyperplasia include growth: with enhanced crypt cell production rates, plus greater villus height and crypt depths (also documented in human infants: McDuffie et al., 2011) that effectively enlarge mucosal surface areas (Lauronen et al., 1998; Drozdowski et al., 2009), along with changes in brush border membrane fluidity/permeability and altered carrier-mediated transport. cDNA microarrays have identified important components of this process including genes regulating cell cycle, proliferation and differentiation and apoptosis (reviewed by Drozdowski et al., 2009). These enteroplastic intestinal adaptations involve various structural and functional changes in response to loss of function and integration of these altered processes represent attempts by the body to attenuate the effects of otherwise restricted nutrient uptake on energy balance.

It is clear that the gut has strong inherent abilities to respond to attempts to limit the function and effectiveness of the tract in supporting nutritional needs of the whole organism, which was even more clearly demonstrated using additive challenge experiments by Hammond (1997) whereby pregnant and lactating rats were subject to 50% resection of small intestine yet demonstrated enteroplastic recovery of mucosal mass to 130% and serosal to 110%. However there was only a disproportionate 30% increase in small intestine length. These results provided some indication of prioritisation, the speed of change possible and demonstrated that very robust maternal enteroplastic adaptation mechanisms are in place, when food is available, to protect the energetic requirements of the combined unit of mother/offspring (dyad). It seems reasonable to assume that, as detailed earlier with the extent of effects experienced from exclusion of entire organs or partial segments of tract, ranging from metabolic complications and deficiencies, to altered gut hormone profiles, that these maternal enteroplastic adaptations are likely to be reflective of more extensive metabolic changes than simply providing compensatory gut hypertrophy in one area.

2.6. Gut barrier integrity and transmigration

The luminal contents are separated from surrounding tissues by a barrier formed by intestinal epithelium cells with tight, adhering and gap junctions and proteins (Puzio et al., 2021) that mediate intestinal permeability to large molecules and microbes. A number of chronic health conditions involving gut and systemic immune functioning, insulin-resistance and metabolic syndrome have disruptions in gut barrier permeability (Fan and Pedersen, 2021). Some gut hormones have anti-inflammatory roles in multiple tissues (e.g. GLP-1 and agonists: reviewed by Jensterle et al., 2019) and GLP-2 has been implicated in control of epithelial cell proliferation and improved gut barrier integrity (Osinski et al., 2022) with increased amounts associated with recovery of tight junction protein expression and distribution in jejunum and colon. Perturbations in gut barrier function have implications for health as alterations in tight junction proteins promoting gut 'leakiness' and thereby transmigration of microbes/microbial products e.g. bacterial lipopolysaccharide toxins within the body via the blood circulation is generally associated with diseased states. However lower levels of this process occurs in healthy hosts to move specific mutualistic bacteria from genus including *Bacteroides, Lactobacilli* and *Bifidobacteria*. During late pregnancy, gut bacteria gene expression change with some populations reaching a stationary phase, raising the possibility of translocation to other body sites (Rodríguez et al., 2021 citing others). Tight junctions between mammary epithelium are more open after birth when leukocytes/dendritic cells are concentrated in colostrum (Rodríguez et al., 2021) and the existence of an entero-mammary circulation of beneficial microbes has been proposed with barrier integrity maintained by increased expression of tight junction proteins (ZO-1, occluding, claudin).

2.7. Large intestine, home of the gut microbiome

Lower down the gastrointestinal tract is the large intestine or colon which transports and stores the mostly undigested materials, plus absorbs the remaining fluids (Neelis et al., 2016). This area has been less well studied for enteroplastic changes although the mucosa is known to have notable amounts of glucagon-like peptide-1 (GLP-1) and peptide-YY (PYY)-secreting L cells (Christiansen et al., 2018). GLP-1 has systemic effects on glycaemic control, delays gastric emptying, reduces appetite and food intake via hypothalamic pathways, restrains body mass gain but also of note, promotes gut structural changes (Davis and Sandoval, 2020).

It was first suggested in the 1920s that a gut-derived substance was involved in pancreatic insulin secretion but not until the 1970s that incretins were identified. Despite GLP-1 having numerous widespread physiological functions, much more research has focused on its role in the pancreas than its main site of production. GLP-1 instigates glucose-dependent activation of beta-cell insulin secretion, promotes tissue mass increases and survival and inhibits glucagon action. It is now considered that pancreatic alpha-cells produce enough GLP-1 locally when metabolically-stressed to influence beta-cell functions (reviewed in Davis and Sandoval, 2020). More recently, large post operative increases in GLP-1 have been implicated, with GLP-2, in the success of bariatric surgeries for resolving type 2 diabetes and promoting body mass losses.

This gut compartment has extensive microbial colonisation of the luminal surfaces. It houses the resident microbiota and as such has been described as a metabolically active organ (Astbury et al., 2015), with its entire microbiome termed our second genome (Gomez et al., 2019). As well as undertaking their own digestive processes, mammalian herbivores and omnivores rely on these commensal microbes to ferment otherwise indigestible plant fibrous materials, carbohydrates and some proteins, to yield beneficial metabolites – notably short chain fatty acids SCFAs - that contribute towards the overall energy balance and health status of mammals. SCFA are energy sources for maintenance, growth, lipogenesis or are metabolised by liver or more peripheral tissues; in humans they can account for 5-10% of the total energy intake (Bergman, 1990). There are additional substances generated from bile acid processing and other sources (Lamichhane et al., 2018: Table 1), some of which act as neurotransmitters and their precursors (Fan and Pedersen, 2021), also branched chain amino acids (isoleucine, leucine, valine) that are involved in regulation of brain function and behaviour (Di Gesù et al., 2021). From the wide range of metabolites generated, the major component consists of a mixture of SCFA - also called volatile fatty acids, VFA - from saccharolytic fermentation: notably acetate, butyrate and propionate and these regulate microbial influences on the host's physiology. SCFA are rapidly absorbed by the host gut epithelial cells and metabolised locally - their presence, or fermentable fibre, can have local actions stimulating mucosal development, with butyrate having faster and more pronounced effects than acetate and proprionate in rats (Bergman, 1990). Butyrate suppresses inflammation in numerous tissues and along with propionate has predominantly anti-obesogenic effects as they initiate signalling, including stimulation of anorexigenic (satiety) gut peptides PYY and GLP-1, as well as leptin; whereas acetate may promote lipid storage by stimulating ghrelin (Fan and Pedersen, 2021). Depending on the species of bacteria present, and their abundance, differing amounts of other substances are produced (e.g. lactic acid by *Bifidobacteria*). Humans on European diets ferment 50-60 g carbohydrate per day to yield 0.5–0.6 mol VFA (total energy value 140-180 kcal; Bergman, 1990).

The human gut is dominated (~75% of total) by two bacterial phyla: Bacteroidetes (e.g. *Bacteroides*) and Firmicutes (e.g. *Lactobacillus, Clostridium, Enterococcus*) and less so by other phyla, although generally including: Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia. This is generally stable in adults although numerous studies have demonstrated that the composition can be altered by different factors. High taxa diversity of bacterial communities and microbial gene richness with a stable microbiota functional core is considered reflective of a healthy gut ecosystem whereas reduced diversity/distorted composition has been repeatedly associated with metabolic dysbiosis including insulin resistance, obesity, gut dysfunction, inflammation and mood disorders (Di Gesù et al., 2021; Fan and Pedersen, 2021). In chronic malnutrition, loss of anaerobic gut microbiota leads to energy harvest and vitamin deficiencies, malabsorption, reduced immune responses to pathogenic bacteria and diarrhoea (reviewed by Fan and Pedersen, 2021). Despite these accepted generalities, it is worth noting: that most studies are still observational so it remains unknown whether these are secondary effects or primary disease-related mechanisms, especially as many humans with metabolic disorders will also have potentially confounding medication-induced effects on the gut; also, there is no agreed definition of a healthy gut microbiota; particularly as individuals have unique relative distributions of gut bacteria at deep resolutions (e.g. structural variation in: strain diversities, growth rates, gene variants) that combine to affect functionality. Furthermore, especially in the context of pregnancy and lactation that potentially have altered gut transit times, prolonged transit may result in increased richness that due to changing faecal composition is not necessarily reflective of what is currently considered a healthy profile (Fan and Pedersen, 2021 citing Falony et al., 2018).

With these caveats in mind, pregnancy in normal and overweight women has been associated with gut microbiota composition and structure shifts (increased population levels: Collado et al., 2008), with a remodelling over the course with significant reduction/loss in alpha diversity (richness; number of different types within individual) and increased beta diversity (difference between individuals; Koren et al., 2012) by the third trimester, that persisted into early lactation (one month post partum) with enrichment of Proteobacteria and Actinobacteria. This included increased representation/enrichment of lactic acid fermenters *Lactobacillus, Streptococcus*, as well as *Enterococcus* in third trimester (Koren et al., 2012). Proteobacteria have obesity and pro-inflammatory associations; the authors also measured increased inflammatory markers in stool samples and proposed that low-grade

inflammation may develop in pregnancy that could drive the microbial dysbiosis observed and contribute towards host adiposity. In another study with only second parity mothers (Ruebel et al., 2021), pregnancy stage did not substantially influence alpha diversity, but beta diversity altered in late pregnancy with maternal gut microbiome phylum level dominated by Bacteroidetes and Firmicutes and at family level by *Bacteroidaceae*, *Ruminococcaceae* and *Lachnospiraceae*. Specific microbial abundances were: decreases in *Akkermansia* and *Lachnospiraceae* (possible gut health markers related to insulin sensitivity and lipid metabolism: Yang et al., 2020; Ruebel et al., 2021) and increases in Actinobacteria and *Streptococcus*. These more recent studies may help explain some of the larger differences found amongst individual pregnant women as they found microbial composition to be altered by pregnancy status, pre-pregnancy BMI and dietary fat intake, indicating maternal obesity-dependent interactions with the gut microbiome.

Yang et al. (2020) undertook a systematic analysis to identify individual factors and some of these are consistent with those of Ruebel et al. (2021) in that there was a greater effect size of maternal pre-pregnancy body mass/BMI than stage of pregnancy on gut microbiome (with greater *Lachnospiraceae, Roseburia, Bilophila*). These findings have important implications as the maternal gut microbiome shapes the infant gut microbiome thus any dysbiosis, if transferred, could impair offspring brain development and influence neurodevelopmental disorders (evidence for mechanisms reviewed by Di Gesù et al. (2021). Aatsinki et al. (2018) used a different approach and divided the pregnant population into separate groups based on whether they had dominance of either Firmicutes or Bacteroidetes. They found that those dominated by Bacteroidetes in mid-pregnancy were associated with reduced alpha diversity and increased gestational weight gain of mothers. Haddad et al. (2022) examined the gut microbiota of women at 6-weeks' post partum and found differences in maternal alpha and beta diversity, with higher *Ruminococcus,* between lactation status (exclusive breast feeding vs. partial human milk feeding), but further analysis also indicated this was likely to be mediated by maternal BMI category.

Pigs have many physiological similarities to humans, in particular their gut microbiota likewise mainly consisting of Firmicutes and Bacteroidetes phyla. Liu et al. (2019) compared two pig breeds with differing digestive abilities, investigating gut microbe composition and also measuring SCFA concentrations during different stages of pregnancy and lactation. There were breed differences: only one with a clear shift associated with pregnancy relative abundance increases, notably of *Coriobacteriaceae* (Actinobacteria phylum), from early to late pregnancy/gestation but with relative stability during lactation (on the same ad lib diets). Whereas bacterial operational taxonomic units (OTUs) indicated gut microbiota structure changes with enrichment of *Christensenellaceae* (Firmicutes phylum) during lactation in both breeds; comparative analysis revealed 36 taxa were significantly different. No individual or total SCFA changes occurred during pregnancy in either breed whereas total SCFA increased at d3 of lactation until d28; mirrored by faecal measurements.

It is not entirely clear what influences these maternal shifts; Koren et al. (2012) have speculated that immune changes at mucosal surfaces are likely to be involved during pregnancy and some may be due to pre-existing BMI status increasing energy extraction from diet. Different sources of experimental data indicate that female sex hormones oestrogen and progesterone may influence microbiota composition variability as both have been associated with diversity changes, notably increases in *Bifidobacteria* and *Bacteroides* (Yeo et al., 2022). Also, inactive oestrogen metabolites in bile can be reactivated by gut bacteria which may represent a host-endocrine-microbe intestinal adaptation to the additional requirements of pregnancy/lactation increasing the bioavailability of required substances (Yeo et al., 2022).

Pregnant women with gestational diabetes mellitus (during third trimester) had elevated glycaemia and disrupted gut microbiota composition (17 OTUs; 13 in women with previous GDM; 5 differential abundance relating to pre-pregnancy BMI) with higher abundance at

phylum (Actinobacteria) and genus (*Collinsella, Rothia, Desulfovibrio*) levels; aberrant composition that persisted until at least 8 months post partum (Crusell et al., 2018). Metabolic disorders including type 2 and gestational diabetes mellitus and obesity are associated with significant shifts in gut microorganism species composition towards 'dysbiotic', notably the ratio of Bacteroidetes to Firmicutes which has a positive association with glucose tolerance (reviewed by Yeo et al., 2022), loss of butyrate-producing taxa, reduced richness, increase in pro-inflammatory bacteria and pathogens (Fan and Pedersen, 2021). It is notable that pregnancy, as a temporary physiological state of elevated metabolism, has a similar microbial phenotype to chronic metabolic diseases with increased abundance of Proteobacteria in third trimester (Koren et al., 2012), but with the clear distinction that this shift may reflect a temporary adaptation to provide for elevated maternal energy demands. Counter to the usual avoidance of research involving pregnant and lactating females, unravelling some of the complexities involving functionality of the gut microbiome, especially in the early/pre-diabetic state, may be more valid (and feasible using non-invasive faecal samples) as these are generally human subjects that stop taking medications (except vitamins, probiotics, oral contraceptives: Crusell et al., 2018).

2.8. Enteroimmune system

Maternal transfer of microbiota and bacterial metabolites with immune functions take place to offspring through different routes, direct to the mammary glands via blood, via maternal tissues during late pregnancy and lactation, as well as physically during birth and in milk during lactation. Gut-associated lymphoid tissues are in constant close contact with dietary components that contain antigenic substances, as well as resident or transient microbes. They distinguish between tolerated and harmful substances, with macrophages and dendritic cells inducing immune responses on challenge, inducing T helper, T regulatory or natural killer cells, as appropriate. As the lactating mammary gland compartment is closely related to the gut mucosa (Rodríguez et al., 2021) and a site of the mucosal-associated lymphoid tissue system, after antigenic exposure in the gut, lymphocytes may migrate to the unexposed mammary mucosal surface, primed for appropriate microbiota transfer. Tight junctions between gut epithelial cells can be opened by dendritic cells whilst preserving the epithelial barrier function and human studies have shown bacterial translocation can take place during late pregnancy and lactation.

2.9. The continuum of enteroendocrine cells, with their far-reaching effects

Despite functional differences throughout the gut compartments travelling from top to bottom, one way the entire gastrointestinal tract is continuous is that it has epithelium from start to finish and a recent study has mapped the entire human gut epithelial layer at single cell resolution – revealing the changing cell types along the tract with full gene expression, although so far the work has only been performed in three males. Donated organs were used instead of the usual limited biopsy samples which allowed localisation of cell types and associated functions to much more exact regions of the gastrointestinal tract than has been possible before (Burclaff et al., 2022). This important first step has revealed unappreciated regional expression and differences suggesting increased potential for differential responses. Many organ/region-specific markers show differentiated lineages, that are unexpectedly enriched, with the authors suggesting that this hints that intestinal stem cells could be transcriptionally primed for organ-specificity and not simply exist in a pan-intestinal state. They have provided differentially expressed genes (DEGs) with transcriptional signatures available for all epithelial lineages in human male small intestine and colon and created an atlas for further study of the potential roles of these cells through the genes that each cell expresses. The next steps are to investigate functional significance in separate areas

of the gut to determine where there are differences or overlaps.

In particular, for a much clearer understanding of how enteroplasticity develops throughout pregnancy and lactation, single cell mapping of the female gastrointestinal tract is needed, focusing on the enteroendocrine cells e.g. L-cells that secrete gut appetite hormones, or alternatively classified by their multiple subtypes as some are polyhormone. Enteroendocrine cells have essential central roles in regulating nutrients, particularly: glucose, peptides and fatty acid absorption in the gut and their loss results in chronic malabsorptive diarrhoea (reviewed by McCauley, 2020). Enteroendocrine cells are distributed throughout the luminal epithelium and respond to acute nutritional flux and signals (including gut microbial) as they reach particular areas of the gut. Enteroendocrine cells secrete over 30 gut hormones and show regional expression: stomach: ghrelin (Johnson et al., 2019; Wellman et al., 2022); small intestine: e.g. cholecystokinin, GLP-2; colon: PYY (Taylor et al., 2009), GLP-1 (Yeo et al., 2022), GLP-2. Gut hormones, notably ghrelin, PYY, GLP-1 and GLP-2 have important roles in metabolic adaptations, maternal glucose handling (Jensterle et al., 2019), lipid handing, trophic actions in the gut but also potentially are modulating signals between reproductive and metabolic axes (Taylor et al., 2009; Johnson et al., 2019; Jensterle et al., 2019), including kisspeptin regulation of GnRH with energy balance changes. Numerous microbially-produced products in the intestine have effects on the entire holobiont including endocrine regulation, whole-body energy homeostasis, adiposity and inflammation, glucose tolerance and insulin sensitivity (reviewed by Fan and Pedersen, 2021). Acetate, butyrate and proprionate act on G protein-coupled receptors GPCR-41/43 on enteroendocrine L-cells to release PYY and GLP-1 that can induce insulin biosynthesis and improve glucose metabolism; acetate can stimulate ghrelin secretion to enhance lipid storage. Secondary bile acids act through TGR5 receptor promoting GLP-1 release. Epidemiological studies suggest that patients that have undergone total colectomies have increased risk of type 2 diabetes mellitus which supports growing evidence of the role of some distal gut hormones and components of the microbiota in whole body glucose regulation (Fan and Pedersen, 2021). Furthermore, hyperglycaemia is associated with a 'leaky' gut due to increased intestinal barrier permeability as tight junction integrity is altered.

There is already realised potential in the form of exogenous peptide treatments, and further potential, for the antidiabetic and antiobesity effects of some gut hormones (Christiansen et al., 2018); GLP-1: Davis and Sandoval, 2020). Some gut hormones are co-secreted in response to nutrient signals and multiple layers of regulation ensure their absorption efficiency roles are optimal by means of a feed-forward loop connecting appetite, digestion and metabolism (Wellman et al., 2022). There are some well-established enteroendocrine cells to enteric nervous system signals controlling gut functions, including blood flow, smooth muscle contraction and motility (affecting transit times). Far less is known about neuroplasticity involved in reorganising enteric innervations with functional changes to regulate/coordinate nutrient absorption (McCauley, 2020; Puzio et al., 2021) although enteroendocrine cells and enteric neurons are closely connected within the mucosa, with enteroendocrine cell receptors localised on enteric neurons. Conversely, hormone release can stimulate neuropeptides from enteric neurons which raises a further possibility of identifying combinations that abnormally increase nutrient absorption contributing towards metabolic disease, such as high-fat diet altering small intestine myenteric innervation with GLP-2 augmented lipid absorption (McCauley, 2020).

2.10. Metabolic disturbances reveal non-uniform remodelling gut plasticity

As being able to study maternal gut architecture and functioning during reproductive events is limited, other research areas have to be mined for relevant findings. Studies of metabolic diseases and medical interventions have revealed sex differences in both responses and recovery as the development and pathogenesis of metabolic disorders are notably influenced by gonadal/reproductive hormones (Hutch et al., 2021). Females with early ovarian insufficiency or after they have reached menopause (hormonally similar in lacking oestradiol), have greater risk factors for developing metabolic dysfunction, in particular type 2 diabetes, with larger adipose depots, hyperglycaemia, increased liver triglycerides; all greater than in premenopausal women. Ongoing oestradiol replacement can reverse some disorders and oestrogen bioavailability appears to be critical for normal tight metabolic control in females and contributes to more successful body mass loss following bariatric surgery.

Type 2 diabetes mellitus is a chronic metabolic disease of elevated fasting circulating glucose and is commonly associated with gastrointestinal disorders. One particularly informative study used experimental streptozotocin-induced diabetes (beta-cell death), albeit again only in males. This treatment provoked double/triple food intake and ensuing morphometric changes and intestinal remodelling (Zhao et al., 2003). The authors documented changes in duodenum, jejunum and ileum, with increases in mass per cm length, tissue wall thickness and cross-sectional areas and longitudinal stiffness with disease progression. Furthermore they measured different layers (mucosa, submucosa, total wall thickness) and found non-uniform remodelling. In the duodenal segment – all layers increased – and opening angle and residual strain were lower in duodenum, but mainly just the mucosal layers were larger in jejunum and ileum compared with controls, so the inner wall grew more than outer wall causing the opening angle to increase and inner residual strain to be more compressive in these segments. Circumferential and longitudinal stiffness of intestinal wall increased with time/disease duration. Increased wall stiffness was thought to be due to thickening of collagen – with potential to alter gut transit and propulsion times. Pereira et al. (2021) used the Goto-Kakizaki experimental rat model of spontaneous and non-obese type 2 diabetes mellitus, similarly finding altered small intestine morphology with local inflammation and 25% slower intestinal transit. In the Zhao et al. (2003) study, not all of the intestinal growth response was due directly to the doubled/tripled food intake - increased plasma/colon tissue GLP-2 was implicated as these elevations preceded the structural changes observed and furthermore could be resolved with insulin treatment. Such an indirect stimulation of gut hormones could occur with larger and earlier delivery of luminal contents thereby stimulating L-cells. Similar studies need to be performed in diabetic females to confirm whether the same or different changes occur as it could be reasonable to predict that more pronounced effects may occur, especially during pregnancy with gestational diabetes.

2.11. Animal models reveal enteroplastic changes to normal pregnancy and lactation demands

Most of what is known about enteroplasticity during normal pregnancy and lactation has been gained from observation or experiments using animal models. Adaptations occur firstly in pregnancy and then again during lactation – some of the pregnancy changes support early lactation, before new homeorhetic changes become effective. During pregnancy there is a dramatic increase in supporting organ workloads, with increased systemic blood flow and volume (Meyer and Caton, 2016) that helps the maternal gut maintain digestive efficiency with elevated amounts processed to ensure adequate absorption and transfer of more nutrients. By the end of pregnancy, the increase in small intestine mass total uptake of glucose and amino acids is higher and the available capacity for total nutrient transport is already 400% higher than intake (Hammond, 1997). This available or excess capacity is then rapidly used up as lactation begins and food intake elevates even further.

Hammond (1997) reviewed rodent maternal enteroplastic intestine changes during lactation, consistently finding cell proliferation and hypertrophy in upper intestinal areas with large increases in small intestine size – masses, lengths and mucosal surface areas. Similar small

intestine adaptations to lactation have also been documented in sheep and pigs (Meyer and Caton, 2016). The increases were mostly mucosal and more specifically in duodenum and jejunum compartments where digestion and nutrient absorption occur. Mucosal mass increases were disproportionately larger than length (which would involve both mucosal and muscular/serosal tissues). There were some inconsistencies between studies in epithelial enzyme activity (tissue mass specific activity) but due to increased mass/total capacity the overall result allows a greater amount of large macromolecule breakdown. Apparent decreases of nutrient transporter rates were thought to be due to increased turnover of cells but had the same overall effect due to increased tissue mass so that total uptake of nutrients was increased during lactation. The general consensus was that both processes occur, allowing for greater total nutrient uptake capacity (macronutrients, vitamins, minerals) during lactation (Hammond, 1997).

A later quantitative metaanalysis by Naya et al. (2007) tested these established observations on gut size flexibility in captive rodents, using lengths and dry mass as their response variables. Digestive capacity upgrading is also known to show increases in these features in response to increased food intakes from other qualitatively different challenges, such as growth (small intestine increased capillary number/area, surface densities, total vascularity in steers) or defensive adaptations to: reduced diet quality and cold environmental temperatures (increased mass: small intestine, liver, kidneys, Speakman 2008). Naya et al. (2007) compared the results of 25 laboratory rodent studies (1934-2004): 55 for small intestine length, 67 for small intestine dry mass, 25 for hindgut (large intestine + caecum) length, and 26 for hindgut dry mass. Their analyses demonstrated that whilst variable adjustments in gut size occur under different circumstances, variation in the amount of digestive flexibility is determined differentially by qualitatively different experimental factors/challenges. Lactation was clearly responsible for stimulating the largest changes (cumulative effect size) in small intestine mass and length, followed by lesser changes in diet quality, pregnancy, and finally, by reduced temperatures. Whereas in contrast, although based on more limited studies, changes in large intestine length (but not mass) were greater in relation to diet quality changes than for the other energy-demanding conditions. Thus their analysis confirmed the earlier ideas and locations of gross changes in gut size flexibility during lactation but by comparing situations with different energy-demands and finding differential responses have also provided support for the idea that there is a hierarchy of demands or prioritisation determining the extent of adaptations that are both experienced and possible. This suggests that more refined mechanisms are available to influence the extent of inducible enteroplasticity.

Hammond (1997) in her review, provided additional evidence that the extent to which small intestine mass increased in mice that accompanied lactation was a function of increased food/nutrient intake that closely correlated with litter size and mass. Increased gut tissue mass accommodated increased volume partly by slowing food passage rate to maintain digestive efficiency and also by a disproportionate increase in mucosal mass/surface area that facilitated increased uptake of nutrients. Naya et al.'s (2007) metaanalysis further confirmed this positive relationship between litter size and maternal small intestine gut mass/length flexibility. These studies highlight that it is important to more closely examine the qualities of a particular type of demand, such as its duration or intensity, not just its presence or absence (Kristan and Hammond, 2006; Naya et al., 2007).

Another factor that can affect the quality of maternal response to energetic demand is whether the dam herself is young and still maturing so nutrients are partitioned towards her own growth at the expense of milk production (dairy cows: Wathes et al., 2007; Meyer and Caton, 2016).

2.12. Hyperphagia – the gut's largest challenge

The progress of lactogenesis is non-uniform, building towards a peak

of demand. Thus the dam's metabolic adjustments remain dynamic, as the size of the response in terms of volume and composition of milk adjusts to the changing demands/requirements of the offspring/litter. Due to the constant outward drain of energy into milk, the continuous replacement of nutrients is required. Hyperphagia - eating (and drinking) substantially more than 'maintenance' - is the biggest driver of maternal gut adaptations and the most noticeable feature of lactation, and to a lesser extent, pregnancy. Food intake increases are variable across species but a human mother's peak milk production requires ~25% increase in energy intake, a lot less than rodents, with up to 400% in rats. To achieve such massive increases, feeding behaviour in terms of meal frequency and amounts increase, stimulated by endogenous hormones and signals integrated with whole body energy homeostasis. Animal studies indicate that energy-sparing activity reduction (Speakman, 2008) can occur when energy replacement is limited to maintain optimal physiological functioning for reproductive tasks but this is less of an issue for humans with easily accessible food sources. Hyperphagia as a strategy needs physical capacity available to hold additional food, then metabolic capacity for its processing. Under normal conditions, the epithelial cells of the gut are acutely responsive to the arrival and direct contact of numerous types of nutrient cues and hormones in the different compartments, with continuous environmental signals influencing intestinal function.

2.13. Experimental manipulation of enteroplastic adaptations

Natural maternal gut modifications have been recorded during different reproductive phases in a range of species from flies (Reiff et al., 2015) to mammals (Cripps and Williams, 1975; Speakman 2008; Johnson et al., 2019) and are considered important adaptations for reproductive success. Enteroplastic adaptation involving stimulation of mucosal hyperplasia is biosynthetically and energetically costly to initiate and then maintain (Karasov et al., 2011) but necessary for expanded capacity to rapidly accommodate to larger volumes of food. The signals and mechanisms controlling physical gut adjustments, and whether they take place or not, remain poorly understood although these mechanisms are likely to involve stimulation of secretion and action of trophic gastrointestinal hormones.

For investigative research studies using small model organisms, a commonly adopted strategy is to standardise litter sizes, using 'normal' - often 8 pups. Our laboratory has used standard 8 litter size rat studies and documented dam body composition changes in the Wistar strain. Dam gross body mass increases were seen during lactation, despite the expected observation that white adipose tissue depots were depleted as lactation progressed, which was confirmed post mortem by extensive gastrointestinal organ tissue expansion and remodelling (Johnson et al., 2019). Using intensive measurements taken at identified key time points throughout pregnancy and lactation, we uncovered more subtle, potentially coordinated organ-specific increases and associated endocrine profile specific changes that had taken place earlier. Under our experimental conditions during only the post partum period, stomach masses had increased the most by late lactation. Whereas caecum mass increases and duodenal, ascending colon and descending colon circumference widening, had all peaked in the day 10 of lactation group – before peak lactation occurs in the rat. Likewise gastrointestinal masses and lengths peaked for small intestine by d10 of lactation but not until day 25, towards the end of lactation period, for large intestine. Small intestine increases and timing were consistent with other studies, as described above. As we had also simultaneously characterised fed and fasted gut peptides in matched samples for total ghrelin, PYY and GLP-1 and based on these profiles, we hypothesised that persistent increases (throughout pregnancy) and early lactation day 5 peak increases in colon tissue PYY and GLP-1 concentrations provided 'permissive' endocrine remodelling signals initiating large intestine hypertrophy (potentially also pancreatic beta-cell mass: Davis and Sandoval, 2020). Additionally these gut hormone increases could have initiated the ileal

brake mechanism, slowing down gut transit times and allowing more absorption and fermentation (via microbial abundance/diversity changes) in the observed enlarged gut chambers.

These maternal gut modifications were recorded under standard litter conditions and would have increased tissue maintenance costs on top of expansion costs (Speakman, 2008). Based on the simple premise that this control group with 8 pups was judged to provide optimal conditions, with some potential buffering capacity and because gut expansion did occur implies there was enough spare capacity to incur increased tissue maintenance costs. Our studies looked into this further with experimental manipulations of litter size to provide overnutrition and undernutrition groups, but otherwise using the same methodology (Johnson et al., 2019). To investigate the effects of under and over nutrition (on the pups) or demand (on the dam), litter sizes were adjusted accordingly. This paradigm challenges the pups and dams metabolically – by restricting nutritional inputs or increasing energetic outputs, but remaining within strict ethical limits to minimise any stress responses.

Dam groups with variable litter sizes under our experimental conditions revealed different types or combinations of adaptations to metabolic demand, under ad lib feeding conditions. Dams feeding only 4 pups had smaller small intestine, caecum and large intestine tissues than dams feeding 8 pups, likely due to reduced suckling demand and sufficient nutrient intake, with no requirement to invest in additional hypertrophy (than had already pre-emptively occurred during pregnancy). The control dams feeding 8 pups had increased descending colon PYY and further increased gut size so energy intake was consistent and sufficient to support and maintain modification of gut tissues and/or increased capacity to extract more nutrients. Whereas despite no increases in gut masses/length in dams feeding the largest litter sizes of 12 pups, as there was insufficient 'spare' energy or time to initiate hyperplasia whilst feeding 12, they did have increased/wider gut circumferences in duodenum, ascending colon and descending colon. These more biomechanical changes may reflect the simplest and least energetically expensive adaptation to quickly accommodate lactation hyperphagia. These dams also had significantly increased circulating blood volumes to dams with 8 or 4 pups. Under our experimental conditions, there was not a simple small intestine mass to litter size effect but distinct combinations of adaptations to varying sizes of energetic challenges. It is also noteworthy that these were all primiparous (first litter) dam studies with young females who would have potentially had restrained responses as they were still growing/maturing.

We made some additional observations from this work with two further groups of dams. As we also ended up with a group of rats whose litters all died, they were opportunistically retained to determine how pregnancy but with no ensuing lactation affected the same measures. Their small intestine/large intestine lengths and large intestine wet masses were all significantly lower than dams with litters of 8. However, these dams without lactation had significantly more retained abdominal cavity white adipose tissue and were markedly resistant to an acute fast prior to culling, compared with primiparous dams with 4, 8 and 12 pup litters (but similar to multiparous, see below). This was only a limited observation but does suggest altered metabolic control in dams without an ensuing lactation and requires further exploration in carefully controlled studies. Any increased lipid mass retention and/or metabolic resistance to mobilise stored lipids could contribute to increased health risks for human females that have never breastfed as they are known to already have a: 30% increased risk for cancer of the ovarian epithelium and 50% increased risk of diabetes (Del Ciampo and Del Ciampo, 2018).

We also made some simple observations post lactation/weaning once the pups were feeding independently and separated for the dam to recover, as we were interested to know to what extent the increased gut capacity adaptations persisted. A further group of multiparous dams (second successfully raised litter), with measurements taken 2 weeks post weaning, had slightly but not fully reduced measures (consistent with Cripps and Williams, 1975), suggesting that some gastrointestinal

tract reduction had taken place, once the suckling stimulus and metabolic demand was withdrawn. However, both these additional groups - multiparous dams (and dams without lactation) had significantly more retained abdominal cavity white adipose tissue and were markedly resistant to (losing body mass) in an acute fast prior to culling, compared with primiparous dams with 4, 8 and 12 pup litters. If these reflect generalised mammalian maternal responses, there may be biological reasons for human females not being able to lose body mass easily after being pregnant (Jayasinghe et al., 2022), especially after multiple gestation periods and/or for those without any energetic drain of lactation. From these initial findings in rats, it remains feasible that some maternal adjustments in gut size and to metabolic control that occur during pregnancy and lactation may persist beyond weaning and/or be further altered by lactation duration and parity. It is not only whether the increased physical size shrinks back – but if metabolic homeorhetic adjustments that are nutrient harvesting reset back but on a higher trajectory than they were previously, or if any changes in appetite regulation or hormone resistances remain. Also, any pre-existing maternal insulin resistance/type 2 diabetes mellitus or obesity may additionally or differentially alter these normal gut adaptations to pregnancy and lactation, which in turn could affect the immediate or longer term metabolic health of their offspring. So if natural adaptive and/or lifestyle/dietary increases are maintained after reproductive activities cease in females, they may inadvertently contribute to unwanted body mass gains and associated metabolic and cardiovascular problems, with increased risk of some cancers (Reiff et al., 2015). It is clear that much more detailed work is required by future studies to further elucidate the adjustment of and buffering capabilities of both gut changes and appetite-regulatory systems during these times, to provide effective preventative measures, guidance and treatments to avoid or limit health complications in both mothers and their offspring.

2.14. Recovery, retention and potential carry over

In the immediate recovery period after lactation, mammary glands undergo regression (involution, Lemay et al., 2007; Jena et al., 2019; or 'drying off' in cows) with tissue exfoliation and epithelial apoptosis that would reduce the amount of cells with mutations, in accordance with reduced breast cancer risks in women who have breastfed (Del Ciampo and Del Ciampo, 2018). The dam also needs to replenish body reserves used during pregnancy and lactation, such as the 4–7% bone loss which is reversed within the year after weaning as compensatory mechanisms increase intestinal and renal absorption and calcium mobilisation (Canul-Medina and Fernandez-Mejia, 2019; Del Ciampo and Del Ciampo, 2018).

Most enteroplastic studies only look for adaptations during the lactation period, not whether they persist afterwards, which paradoxically has potentially much more health relevance for humans. Where intestinal length increases have been recorded during lactation in rats, maternal adaptive increases diminished by day 30 (after weaning) but did not completely regress to pre-gravid (Cripps and Williams, 1975; Hammond, 1997; Johnson, 2015). After lactation ends, how and to what extent do homeorhetic changes revert to previous homeostatic set points or do they remain at new ones? Any carry over and persistence of earlier reproductive enteroplastic changes risk cumulative increases in body mass (Jayasinghe et al., 2022) through actions of energy-harvesting gastrointestinal machinery leading to excess storage, with increased metabolic dysfunction and cancer risks; these become particularly important post menopausally. A recent study has provided evidence that gut changes can still be induced in older females: de Fátima Laureano Martins et al. (2022) investigated the potential beneficial effects of Yacon a native Andean root (*Smallanthus sonchifolius*) at 6% fructo-oligosaccharides (FOS)/inulin on intestinal health as its fermentation by *Bifidobacterium* and *Lactobacillus* generates lactic acid and SCFAs. Its consumption before and after reproductive aging led to less weight gain in this animal model, increased GLP1-immunoreactive cells

as well as generating caecum, ileum and colon crypt hypertrophy and increased lactic acid in caecal content. This suggests that dietary induced enteroplasticity has potential to be manipulated in the post reproductive period - using specific foods to control aspects of microbiota functions and manage excess body mass gain for females to minimise longer term health risks.

2.15. Summary: enteroplasticity of lactation framework: providing a hierarchy of options to test

Mammalian body size is closely related to and reflective of overall gut capacity and nutrient/energy requirements, although interactions between food intake and time resident in the gut is still argued to be potentially more important for larger mammals (Clauss et al., 2007). Gut capacity describes the potential to hold ingested food substances that move along the gut undergoing digestion, fermentation, uptake of released nutrients in specialised areas, etc, as covered earlier. Other biomechanical properties of the longitudinal stretches of tissues involved can either limit transit time or extend food residence time (mean retention time) within the gut, affecting many processes (Clauss et al., 2007). Phenotypic flexibility enhances an organism's ability to adapt - providing more resources for specific purposes such as development, growth and reproduction (Naya et al., 2007). Only modifying the amount needed when there is an existing functional demand and only for as long as required is the most energy-efficient solution. Enteroplasticity as a term currently encompasses a whole host of different situations and potential adaptations of digestive and metabolic features, some structural and others biomechanical, but all contributing towards the combined goal of increasing immediately available energy (or to preserve limited) to serve the dominant need. Where excess energy is not available to physically increase and maintain additional tissues, simpler adaptations can be adopted instead to reduce transport times of nutrients through the gut.

To borrow a phrase: *biological systems as a whole embody solutions to important biological challenges* (Sundrum, 2015) so only there are the answers likely to be found. A clearer understanding of the physical and mechanical intestinal adaptations and their interactions with metabolic processes is needed from more comprehensive focused research studies in females to provide better explanations of how organisms can exceed expectations during periods of energy demand/metabolic stress such as lactation. The largest unanswered question remains – do these adaptations take place in humans? If so, to what extent and how long do they persist? Assuming some do and potentially repeatedly with multiple gestations, findings could be harnessed to provide improved strategies for pre and postnatal maternal health promotion guidance to avoid inadvertent additional harvesting, processing and storage of nutrients to minimise the negative cumulative health risks. For lactation, the key distinctive feature that contributes to maintaining immediate health and minimising future health risks is that excess (to normal requirement) ingested and/or mobilised energy and nutrients are neither fully metabolised nor retained as they leave the maternal body disguised as milk.

Many traits of the gastrointestinal tract are phenotypically flexible to adjust digestive performance, but the degree of flexibility depends on many complex interactions between the energetic challenge and nutrients available. They do appear to be based on some similar patterns of occurrence, seen across different species and conditions - implying common underlying mechanisms. Based on all the disparate sources of information reviewed, a general framework for enteroplastic adaptations to lactation, is proposed and left open for future testing.

Many mechanisms are involved, although responses and limitations can be broken down into steps:

- The extent and duration of adaptive response(s) is firstly driven by *where* the energy flux is directed towards - the energy-demanding factor, for example:

  o external (prioritisation: milk – offspring food source; exercise/ activity - physical effort; heat generation – protection from cold), restrained by
  o internal (energy-sparing due to starvation/undernutrition/fasting; partitioned towards maturation/growth; excess energy - storage/ growth)
- A priority hierarchy determines the dominance and extent of the adaptation in individual organs (e.g. small intestine: lactation > diet quality > pregnancy > cold; other organ mass increases involved).
- Where the internal support comes from is driven by immediately available internal reserves (e.g. lipid depots) and whether there are constantly provided external inputs (e.g. food via hyperphagia) that can be transformed quickly ready for use.
- Starting body composition and individual characteristics (including features of microbiota) determine energy balance status and responses.
- The qualities of a demand - intensity and duration - determine the *extent* of adaptation adopted:
  o homeorhetic shifts occur to serve immediate high priority energy demands, especially whilst in negative energy balance. Shifts may return to their previous or a new trajectory afterwards;
  o changes are not uniform across gut compartments;
  o changes can be progressive i.e. occur step-wise and may need synchronised timing of available energy for maintenance with trophic hormone stimulation to manifest in physical growth;
  o increases in energy demand mainly/firstly affect the size of the small intestine;
  o changes in the amount of undigestible material in the diet affect the size of the large intestine or fermentative chambers but also potentially the microbiota abundance/composition (further confounded by existing metabolic/BMI status).
- Energy-demanding factors (within their hierarchy of importance) determine different magnitudes of enteroplastic adaptation within limits of additional influences, e.g.:
  o some energy-demanding factors can elicit additive increases e.g. lactation and cold;
  o different species requirements (e.g. range of offspring produced/ litter sizes);
  o maternal maturity: nulliparity (if not fully physically mature, some nutrient partitioning remains towards maternal growth); primiparous and multiparous dams may already have pre-existing maternal adaptations from earlier reproductive periods.
- Mammalian gut size increases in parallel with increased food consumption (hyperphagia):
  o extent of increase determined additionally by litter size; although also limited by feed intake quantity and quality;
  o organs/compartment masses: progressive increases are observed throughout pregnancy and lactation in stomach, liver, small and large intestines. Increases in adiposity late gestation, decreases in adiposity with lactation;
  o changes/increases in gut hormones with trophic properties e.g. PYY/GLP-1/GLP-2 signal potential to initiate further growth in large intestine compartments later in lactation.
- Where energy to sustain any physical adaptations is limited/not available, less structural adaptations occur and more biomechanical changes may be implemented instead:
  o gut transit/mean retention times lengthened, ileal brake slows content passage to maximise nutrient extraction, microbiota diversity may increase;
  o reflected by increased gut lumen circumference/diameters, more stomach/caecal contents, increased blood volume, increased body temperature.

Metabolic dysfunction on the other hand can cause non-uniform remodelling; it is hard to reconcile if this is an 'appropriate' adaptation to metabolic challenge or simply aberrant. Gastrointestinal

dysfunction or non-homogenous modifications affecting mechanisms such as the ileal brake, slowing gut content transit to facilitate nutrient harvesting, could be behind some of the 'minor' unpleasant common gastrointestinal complaints of pregnancy and lactation including: dysphagia, acid reflux, nausea, vomiting, satiety issues, abdominal pain, constipation and diarrhoea.

It is finally worth briefly considering insights gained from the alternate clinical situation, where surgical attempts are employed to restrict gut capacity and reduce efficiency of uptake of nutrients involving bariatric surgeries (Seeley et al., 2015). Differences in intestinal L-cell numbers/hypertrophy occur in separate studies and are likely to vary with whether mechanical and/or morphological adaptations take place in response to alternative surgical procedures but related to faster/increased nutrient delivery to the distal gut which is considered sufficient stimulus to induce the approximately 10-fold increase in postprandial GLP-1 concentrations (reviewed by Davis and Sandoval, 2020). Surgical rearrangement of the gastrointestinal tract can take many forms and despite mechanisms of how it works not being fully understood, beyond GLP-1 improving insulin secretion and sensitivity, bariatric surgeries are currently the most effective treatment for rapidly reducing metabolic dysfunction to resolve type 2 diabetes mellitus and lose excess body mass in obese and diabetic individuals; also to restore fertility. Male and female animals both see expected metabolic and body mass (loss) improvements although ovarian hormones seem necessary for the full impact as menstruating/cycling women lose more body mass in the two years following surgery than those that are post menopausal. However, in pre-clinical animal models fed high fat diets post-surgery, females were more resistant to liver lipid decreases and corresponding differences are found in human hepatic gene expression key regulators of lipid metabolism and inflammation (reviewed by Hutch et al., 2021). These observations emphasise the importance of appropriate post-surgical diets to achieve maximum benefits in the same way that there needs to be improved post lactation nutritional guidance for females.

The increasing uptake of bariatric surgery has particular relevance relating to female health impacts as they form the largest (over 80%) proportion of cases, with half of these now being in women of reproductive age. Whilst the immediate health benefits are substantial, with improvements in body mass reduction, glucose and liver lipid metabolism, there can also be some serious negative side effects. Pregnancy and lactation outcomes following bariatric surgery in pre-clinical animal studies have been reported (reviewed by Spann and Grayson, 2020) including offspring with intrauterine growth restriction, born smaller and shorter. Lactation following vertical sleeve gastrectomy had a milk caloric content shift, with total calories from fat reduced and total calories increased from glucose, changes with unwanted negative metabolic consequences for reliant offspring, especially if they needed compensatory catch-up growth (Hutch et al., 2021). Robust physiological sex differences in metabolic responses to bariatric surgeries and interactions with diet suggest the mechanistic pathways are dependent on different hormone profiles and warrant urgent further exploration.

2.16. Final thoughts

Biological evolutionary history, with more recent epidemiological and clinical studies provide overwhelming evidence and confirmation that mammalian breast feeding provides short- and long-term health benefits for individuals and communities (Del Ciampo and Del Ciampo, 2018). With the focus turned to the mother, it is becoming increasingly apparent that lactation has an important role in maternal recovery from pregnancy and the outcomes of recovery from both reproductive phases can influence many future health risks.

Physiological costs to small mammals and large mammals are different (Speakman, 2008). Smaller mammals have higher metabolic rates and generally have larger litter sizes so their short-term increased requirements are relatively more dramatic, as are their potential intestinal structural changes, the magnitude of which can increase with litter size. Whilst these may not mirror exactly what occurs or is even possible in larger mammals, the changes manifested in small mammals from experimental and pre-clinical investigations are informative (Hammond, 1997). It is unlikely that modern humans with steady food supplies are working close to their physiological capacities, in contrast to subsistence cultures and wild animal populations where limited food supplies will be reflected in higher mortality rates. But at the other extreme, increasing numbers of humans are overweight/obese from a younger age and increasingly more are subject to surgical procedures or hormonal appetite suppressants (Jensterle et al., 2019) prior to reproducing. This only looks set to continue so pregnancy and lactation with additional enteroplastic adaptations that promote nutrient harvesting may become counterproductive to female health and offset the existing health benefits of lactation.

Data availability

This is a review article

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