Microbiome impact on metabolism and function of sex, thyroid, growth and parathyroid hormones

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INTRODUCTION

By definition microbiome is the catalog of commensal bacteria (microbiota) and their genes associated with host (Ursell et al., 2012). Human microbiome is strongly associated with the number of processes, which occur in host organism, affecting its immunological, nutritional and metabolic functions. That is why in recent years the conception of human-microbiome superorganism was proposed (Dietert & Dietert, 2012). The microbial composition is established in early life and is mainly dependent on mode of delivery and diet. After infancy the main factors which affect microbiome are diet, antibiotic treatment, obesity, sex and geography. There are complex bidirectional relations between host-endocrine system and bacteria, which justifies the use of term “human-microbiome superorganism” also in hormonal system and bacteria (Macdonald et al., 1983). Moreover steroids undergo enterohepatic circulation (EHC).

Large number of species found in human intestinal or other microbiomes, such as Eubacterium lentum, Bac

SEX HORMONES AND REPRODUCTION

Steroid sex hormones such as estradiol (E2), testosterone (T) and progesterone (P4) modulate a number of physiological processes, both related and unrelated to reproduction. They are produced in endocrine glands such as adrenal cortex, ovaries and testes as well as in peripheral tissues like skin. They act through intracellular and extracellular receptors and affect e.g. host blood pressure, energy homeostasis and metabolism, bone remodeling, mood, erythropoiesis and cells growth. Then they are metabolized in the liver and excreted with the bile. Thus, liver metabolism of steroid hormones includes: reduction of the sterol ring A, conjugation with sulfate or glucuronide, and excretion in the bile. Afterward these compounds are deconjugated by bacterial sulfatases (Van Eldere et al., 1988) and lipopolysaccharide (LPS). The first way concerns steroid and thyroid hormones, which are metabolized in the liver and excreted with bile. A large number of intestine bacteria are capable of hydrolysis of hormone conjugates and afterward modify chemical structure of free molecules. Another important aspect concerns the ability of microorganisms associated with plants to produce phytohormones and hormone-like substances, which may modify host metabolism (Tsavkelova et al., 2006).

Abbreviations: E2, estradiol; EHC, enterohepatic circulation; GH, growth hormone; hCG, human chorionic gonadotropin; hPL, human placental lactogen; HSD, hydroxysteroid dehydrogenase; IL, interleukin; LBP, LPS-binding protein; LPS, lipopolysaccharide; P4, progesterone; PAH, polycyclic aromatic hydrocarbons; PTH, parathyroid hormone; SCFA, short chain fatty acid; T, testosterone; TLR, Toll-like receptor
LPS-DEPENDENT EFFECTS

Not to be underestimated is bacterial impact on host steroid hormone metabolism and function which is exerted via LPS (endotoxin). LPS is a part of Gram-negative bacteria cell wall and is a trigger of septic shock. Nevertheless, Marshall stated that LPS is “not less an endotoxin than an exohormone” (Marshall, 2005). He pointed out some LPS features similar to human hormones: 1. its exposure arises from endogenous stores (commensal, Gram-negative bacteria of the gastrointestinal tract), 2. it has a dedicated carrier protein, 3. it interacts with specific cellular receptor, 4. its signaling is specifically modulated by endogenous mechanisms and thus hinder proper reaction to pathogen invasion.

Stereoidal, Bifidobacterium sp., Streplococcus sp. possess enzymes which are involved in degradation of unconjugated steroids, like 21-dehydroxylase, 17,20-desmolase, 16-dehydratase and various dehydrogenases (Macdonald et al., 1983). Interestingly, hydroxysteroid dehydrogenases (HSDs) which are involved in the production of steroid hormones and regulation of their receptor-active and receptor-inactive derivative levels in host cells, were found in bacteria. Accumulating evidence indicates that members of the normal human gastro-intestinal flora (especially members of Actinobacteria, Proteobacteria and Firmicutes), possess HSDs, which are active on keto- or hydroxyl-groups at positions C3, 7, 12, 17 and 20 of steroid compounds (Kisiela et al., 2012). It follows that bacterial HSDs may influence concentration of active steroid derivatives which return to blood throughout EHC. Furthermore, bacterial steroid metabolism has its local long-term effects, since it is known that increased number of bacterial strains capable of steroid degradation in colon is associated with higher risk of colon cancer (Debas, 1981).

Except of direct metabolism of steroid hormones, bacteria may change expression of host genes involved in steroid hormones metabolism and function. Some studies highlight the effects of oral probiotic supplementation on sex hormones metabolism and function. Research conducted on female zebrafish fed with Lactobacillus rhamnosus revealed an increase of transcription of aromatase cytochrome p 19 (cytp19a), vitellogenin (vtg), α isoform of the estrogen receptor (erα), luteinizing hormone receptor (lhr), 20-β hydroxysteroid dehydrogenase (20β-hsd), membrane progesterone receptors α and β and activin βA1 genes. These changes of respective genes’ expression affect sex differentiation during larval development and improve fertility in adults (Carnevali et al., 2013). In turn metabolic analysis with use of murine thyroid infection model revealed that during Salmonella typhimurium infection the 3β-HSD2 and 17β-HSD2 gene expression in the liver is repressed (Antunes et al., 2011). During acute shigellosis in human volunteers, significant reduction in fecal steroid metabolites was observed (Huang et al., 1976). Changes in steroid metabolism during infection may diminish the HPA axis function and thus hinder proper reaction to pathogen invasion.
players in the hormonal function of testes — express TLR-2 and TLR-4, similarly as testicular macrophages (Winnall et al., 2011). In the response to LPS, these latter cells start to produce reactive oxygen species (ROS) and nitric oxide, which alter the function of LC mitochondria (Pomerantz & Pitelka, 1998; Allen et al., 2004). Furthermore, LPS increases NF-κB pathway activity in LC, hence reducing testicular Cyp11a, StAR and 3β-HSD protein levels. In consequence, plasma T level decreases. This effect is partially dependent on LH secretion reduction and inflammatory cytokine level elevation caused by LPS. In SC, LPS acts via MyD88 pathway and activates expression of IL1, IL6 and activin A. In turn, FSH increases cAMP level and promotes lactate, transferrin, stem cell factor (SCF) and inhibit B expression. Intriguingly, these two pathways have probably reciprocal inhibitory effects (Hedger, 2011). It is well established that acute and chronic bacterial infections may be associated with temporary or constant infertility. Vitamin K may contribute to inhibition of inflammatory pathways in testis and may help maintain steady levels of T (Takumi et al., 2011).

NON-LPS-DEPENDENT EFFECTS

Estrogens

Apart from LPS-dependent influence on estrogen function, bacteria may also directly metabolize them and similar particles, like phytoestrogens. To better understand these processes we describe host estrogen metabolism first.

Before excretion with bile, estrogens are metabolized in several steps in the liver (Fig. 1). First of all, intercon-vertible primary estrogens estrone (E1) and estradiol (E2) are hydroxylated by cytochrome P450 enzymes, yielding catechol estrogens: either 2-hydroxy (2-OH) or 4-hydroxy (4-OH) metabolites. Another derivative is made by hydroxylation at C-16α position yielding 16α-hydroxy (16α-OH) derivative. Both 16α-OH and produced in small portion 4-OH derivatives have greater estrogenic activity compared to 2-OH, thus domination of C-16α pathway has been hypothesized as a potential risk factor of breast cancer. However, recent studies do not support this hypothesis (Eliassen et al., 2008). Catechol estrogens might be oxidized into harmful derivatives—quinones—

Figure 1. Metabolism of estrogens and estrogenic compounds.

The bold font denotes the metabolic pathways dependent on bacterial activity. Details, see main text. COMT, catechol-O-methyl transferase; DHT, dihydrotestosterone; ER, estrogen receptor; E, estrogens; E1, estrone; E2, estradiol; E3, estriol; FO3, fructooligosaccharides; LCS, lariciresinol; MAT, matairesinol; O-DMA, O-desmethylangolensin; PRS, pinoresinol; SDG, secoisolariciresinol diglucoside; SHBG, sex hormone binding protein.
that can react with DNA to form deaminating adducts, thus are potential breast cancer initiators (Cavalieri et al., 2006). Methylation of 2-OH and 4-OH estrogens by catechol-O-methyltransferase (COMT) prevents the formation of estrogen quinones, therefore the metoxy and catechol estrogen proportions may influence the cancer risk. Another metabolic pathway includes E2 conversion to 16-ketoE2 and further epimerization to 16-epi-E3 and 17-epi-E3 (Jobe et al., 2013). This latter steroid is very interesting, since it has an anti-inflammatory, but not glycoenic activity (Latman et al., 1994). The phase II of estrogen metabolism includes either glucuronidation or sulfation or methylation, which facilitates its elimination from the organism via bile in feces and with urine.

Plottel and coworkers proposed the term “estrobo- lome” to define the aggregate of enteric bacterial genes whose products are capable of metabolizing estrogens (Plottel & Blaser, 2011). Every individual has its own, unique estrobolobe, which influences EHC of estrogens. Bacterial β-glucuronidases hydrolyze glucuronides which are excreted via bile, thus promotes recycling of aglycone forms through enterohepatic cycle. Excess of β-glucuronidase activity may be associated with higher estrogen-dependent cancers risk. Most species with β-glucuronidase expression are members of Firmicutes phylum and Enterobacteriaceae family (Lactobacillus, Strepto- coccus, Clostridium, Lactobacillus, Roseburia, Faecalibacterium, Eubacterium and Escherichia), however bacteria capable of β-glucuronidase production are also among Actino- bacteria (i.e. Bifidobacterium dentium) (Globus et al., 2011). Administration of oral oxtetracyclin diminished urinary excretion and increased losses of conjugated estrogen metabolites in feces in men, probably by destruction of β-glucuronidase-producing microflora and the interruption of the estrogen recycling (Hämäläinen et al., 1987). In one study fecal β-glucuronidase activity and diversity of fecal microbiome were found to be directly associated with higher concentrations of systemic estrogens (Flores et al., 2012a). Lactic acid bacteria supplements and high-fiber diet (like vegetarian) decrease fecal bacterial β-glucuronidase, which leads to increased fecal excretion and a decreased plasma concentration of estrogen (Goldin et al., 1980, 1982; Goldin & Gorbach, 1984; Han et al., 2005). Other factors which diminish β-glucuronidase activity include oral antibiotic treatment (Hämäläinen et al., 1987), lactic acid bacteria probiotics (Goldin & Gorbach, 1984), high-fiber diet (Grobach, 1984), cellu- lose-fructooligosaccharides (FOS) diet (Gudiel-Urbano & Gofi, 2002), cabbage and sweet pepper pectin protein complexes (Borisenkov et al., 2011), calcium-D-glucarate (Zółtašek et al., 2008), silymarin (Kim et al., 1994), ascorbic acid (Young et al., 1990) and yoghurt (de Moreno de LeBlanc & Perdigon, 2005). In turn bile salts enhance β-glucuronidase activity (Fujisawa & Mori, 1996), while weight loss elevates its fecal level (Flores et al., 2012b). Advanced age and childhood are associated with higher β-glucuronidase activity than middle age, prob- ably due to microflora composition changes throughout life (Goldin & Gorbach, 1977; Mroczynska & Libudzisz, 2010).

In addition to the ability of deconjugation of steroid hormone glucuronides, intestinal bacteria are also able to interconvert steroid derivatives. Alactogenses facialis, Pseudo- domonas aeruginosa, Staphylococcus aureus and Bacillioides fragi- lis interconvert E2 to E1 and participate in regulation of E2 concentration (Järvenpää et al., 1980). Other reactions performed by intestinal bacteria include: E1 formation from E1-3-sulfate, estriol (E3) from 16z-OHE1, 16-ke- toE2 reduction to 16-epiE3 (Macdonald et al., 1983).

Ampicillin treatment diminishes reductive metabolism of estrogens in intestine, thus increases E1/E2 and E2/E3 ratio in both urine and feces (Adlercreutz et al., 1984). Catechol estrogens 2-OHE1 and 2-OHE2 are intercon- verted by human fecal bacteria in anaerobic and aerobic conditions (Järvenpää et al., 1980). Fecal flora is capable of formation of catechol estrogens from metoxygen- gens. It follows that bacteria are able to convert biologically inactive estrogens into active forms (Axelson & Sjövall, 1983).

Gingival bacteria, similarly to the intestinal ones are capable to metabolize sex hormones (García-Gómez et al., 2013). During the pubertal period gingivitis rate signifi- cantly increases and this effect is thought to be relat- ed with higher concentration of steroids in saliva, which may be the carbon source for gingivitis-related bacteria as Prevotella intermedia. This species uptakes estrogens and P4, when its important growth factor, vitamin K, is unavailable (Kornman & Loesche, 1982).

Bacterial colonization of germ-free mice led to the normalization of estrous cycles and increase of repro- duction, probably due to direct influence on intravaginal epithelial cells and indirect effect via EHC of estrogen metabolites (Shimizu et al., 1998). The bidirectional connection between vaginal microflora and estrous cycle was also the conclusion of other studies (Minami et al., 1987).

Van Wiele et al. showed that colon microbiota are able to bioactivate ingested polycyclic aromatic hydro- carbons (PAHs) like naphthalene, phenanthrene, pyrene, and benzo(a)pyrene to estrogenic hydroxylated metabo- lites (Van de Wiele et al., 2005). That conversion can exacerbate cancerogenic effect of PAHs and affect en- docrine system; nevertheless the real role of estrogen PAHs is still unclear (Gozgit et al., 2004).

Another group of exogenous molecules with estrogenic activity are plant-derived phytoestrogens. Lignans and isoflavones are the best studied groups of phytostro- gens and are of interest of this article, because their activity is partly dependent on bacteria.

Flaxseeds, sesame seeds, soybean, berries and nuts are known lignan-rich foods. Lignans are classified as phytoestrogens, despite their lack of biological activity per se. Intestinal bacteria in the upper part of the large bowel convert lignans (secoisolariciresinol, pinoresinol, matairesinol, lariciresinol) to compounds with estrogenic activity called enterolignans: enterolacton (ENL) and enterodiol (END). Enterolignans induce the production of sex hormone-binding globulin (SHBG) in the liver and inhibit aromatase activity, therefore reduce the levels of free and total estrogens in circulation (Hall, 2001). Both ENL and END act as ERα agonist, however they have weaker agonist activity than endogenous estrogens (Carreau et al., 2008). When endogenous estrogens level is low, ENL and END increase total systemic estrogenic effect, whereas when estrogens level is high they prevent estrogen from exerting its effects. Flaxseed-derived enterolignans de- crease proliferation fraction in prostate cancer (Azzad et al., 2013) and risk of colorectal adenoma (Kuijsten et al., 2006). They may increase survival of postmenopausal breast cancer patients (Buck et al., 2011) and lower risk of ER+/PR+ breast cancer (Touillaud et al., 2007). However, other studies did not show associations between enterolignans and lower breast cancer risk (Peeters et al., 2003; Zaineddin et al., 2012). The main lignan, secoisolaricires- inol (SECO) and its diglucoside (SDG), pinoresinol (PRS) and lariciresinol (LCS) are converted into enterolignans via multistage route to ENL, whereas matairesinol undergoes direct conversion to ENL (Wang et al., 2010). END may then be reversibly converted to ENL. The final ENL
to END ratio depends on type of food and individual composition of gut microflora (Bartkiene et al., 2011). In one study the subdominance of ENL-producing bacteria in gastrointestinal tract was found (Eeckhaut et al., 2008). High colon concentrations of Peptostreptococcus productus, Eggerthella lenta and Clostridium cocoides, which are able to demethylase and dehydroxylate SECO are associated with higher serum enterolignans level (Clavel et al., 2005). To conclude, microflora composition affects bioavailability of enterolignans and therefore its action.

Human intestinal flora metabolizes also another class of phytoestrogens, isoflavones in similar to lignans way. *Fabaceae* is the plant family, which members (e.g. soybean) almost exclusively produce isoflavones. Dietary isoflavones occur in the form of glycosides: genistin, daidzin and glycitein, which are bioactivated by bacterial β-glucosidases in colon to aglycones: genistein, daidzein and glycitein. Daidzein is further metabolized to S-(−)-equol and O-desmethylangolensin (O-DMA). Interestingly, not all people are able to produce these derivatives - the first can be found only in 20–60% (so called “equol-producers”) and the latter in 80–90% of the population (Atkinson et al., 2005). Some studies indicate that ability to produce these metabolites, especially equol, is associated with lower risk of breast and prostate cancer, acne and male-pattern baldness (Lund et al., 2004). Intestine-derived S-(−)-equol is a selective ERβ agonist and antiandrogen. Mechanism of its antiandrogenic effect is associated neither with 5α-reductase inhibition nor with binding to androgen receptor, but rather with direct binding to dihydrotestosterone (DHT) (Setchell et al., 2005). Equol non-producers have probably higher risk of some diseases (e.g. prostate cancer) than equol-producers (Akaza, 2012). It was demonstrated that supplementation of equol-producing bacteria may convert non-equol producer into an equol-producer (Decroos et al., 2006). *Lactobacillus garvieae* is used to produce S-(−)-equol rich substance, called SE5-OH (Yee et al., 2008). Supplementation with this food ingredient improved mood in perimenopausal/postmenopausal equol non-producers (Ishiwata et al., 2009). There are some differences in estrogen metabolism by fecal bacteria between equol-producers and non-producers. The latter are more likely than former to convert E1 to E2, and l6α-OHE1 to E3 (Atkinson, 2004). Increased E2 and E3 formation may have mutually exclusive effects in evaluation of breast cancer risk.

Interestingly, amongst species capable of soy milk isoflavone bioconversion are lactic acid bacteria which are also potentially able to influence the renin-angiotensin hormonal system (Yeo & Liong, 2010). These bacteria have special proteolytic enzymes and produce angiotensin- I-converting enzyme (ACE) inhibitory peptides from milk proteins. Inhibitory peptide mixtures are resistant to digestive enzymes and dairy processing (Gobbetti et al., 2000). Significantly lower pressor effect after intravenous angiotensin I injection, was observed in rats which were pre-fed with milk fermented using certain strains of *Lactobacillus helveticus* (Fuglsang et al., 2003).

**Androgens**

*B. fragilis* reversibly reduces 17-keto group of A4 to a 17β-hydroxy derivative — T (Winter et al., 1984b), whereas a steroid-inducible 17α-HSD, capable of converting T to epitestosterone (epiT) was isolated from *Escherichia sp.* VPI 12708 (de Prada et al., 1994). It is possible that intestinal microbiota are capable of epimerizing T to epIT, when both activities are present. EpIT is a hormone which regulates some androgen-dependent action and inhibits 5α-reductase (Stárka, 2003). Pathways which contribute in T-epiT interconversion were not conclusively identified in human, so one cannot exclude significant role of microbiota in this process (Bellemare et al., 2005). These findings suggest possible role of microbiota in the regulation of T level and release of excessive androgens in humans (Donova et al., 2005).

*Clostridium scindens* belongs to the small number of intestinal bacterial species capable of bile acid 7α/β-dehydroxylation, which leads to formation of secondary bile acids (Winter et al., 1984a). Furthermore, *C. scindens* possess steroid-17,20-desmolase which converts corticosteroids to androgens (Bokkenheuser et al., 1986; Kraft et al., 1987). In this process cortisol is transformed into 11-β-hydroxyandrosterone (11-OHA4), which is reabsorbed into the bloodstream and excreted in the urine (Ridlon et al., 2013). Since its discovery in 1953, 11-OHA4 has been a molecule of unknown biological significance. 11-OHA4 has insignificant androgen activity, nevertheless, in accordance with last studies, its derivatives are important metabolites in formation of novel androgens (Bloom et al., 2013). The main source of 11-OHA4 in humans is adrenal cortex, but intestine-derived 11-OHA4 may be also the important source of androgens precursors.

Studies conducted in nonobese diabetic (NOD) mouse model of type 1 diabetes (T1D) showed that in germ free (GF) environment gender bias in T1D had diminished (Markle et al., 2013). Relative to GF males, specific pathogen free (SPF) males had significantly higher levels of T. Interestingly, after transplantation of microbiota from SPF males to females, recipient T levels had elevated and T1D morbidity had decreased. Similar results were observed in another study (Yurkovetskiy et al., 2013). These findings indicate a key role of microbiome in determining host T level and sex differences in susceptibility to autoimmune diseases. *Lactobacillus reuteri* in drinking water prevents ageing male mice from age-related testicular atrophy and elevates theirs T level, probably acting on hypothalamic-pituitary level (Poutahidis et al., 2014). Authors of this study theorize that microbial impact on host T level may favor evolutionary success for the microbe and mammalian host. In general, GF compared to conventional mice have significantly lower weights of testis, epididymis, ductus deferens, kidneys, and adrenals (Fujimura et al., 1990).

Gingival pathogens, *Aggregatibacter actinomycetemcomitans*, *P. intermedia* and *Porphyromonas gingivalis* are capable of reducing T to DHT and increase DHT synthesis by fibroblasts (Soory, 1995). *Bacillus cereus* and *Strptomucobacterium mutans* also possess 5α-steroid reductase activity and moreover, 3β-, 17β- and 20α-HSDs, which allows them to metabolize T and P4 within easy reach in the gingival tissues. During inflammatory periodontal disease, local elevated DHT level foster fibroblast metabolism and matrix production, thus has an impact on inflammatory repair. On the other hand, DHT influence bacterial metabolism, promoting gene expression which facilitates survival and dissemination of bacteria (Markou et al., 2009).

Thus, we propose a new term, analogous to estrogenome, the “androbolome”. Detailed analysis of bacterial genes involved in androgens metabolism is necessary to understand a role of microbiome in androgen-dependent conditions like baldness, acne and prostate cancer.

**Progestins**

*C. innocuum* synthesize the 3α-5α-reductase and *C. paraputrificum* the 3β-5β-reductase which are involved in in-
activation of natural and synthetic progestins by reduction in the ring A (Bokkenheuser et al., 1983). Nevertheless, synthetic progestins, which are used as contraceptives, are much more resistant to reducing enzymes than natural analogs.

Reports on the impact of concomitant use of oral contraceptives and antibiotics on contraception effectiveness are contradictory (Weisberg, 1999; Dickinson et al., 2001; DeRossi & Hersh, 2002; Toh et al., 2011). Rifampicin induces hepatic microsomal enzymes, thus facilitates oral contraceptive (OC) inactivation, whereas other commonly used antibiotics have neither pharmacokinetic nor pharmacodynamic interactions with OC. Probably rare cases of failure of OC associated with antibiotic use are caused by reduction of some microbial populations with β-glucuronidase activity and substantial loss of EHC of hormones.

As mentioned before, LPS reduces placental endocrine function. LPS administration to pregnant mice and ascending intrauterine infection in pregnant rabbits results in rapid P4 decrease and preterm parturition (Fideli et al., 1998).

**THYROID HORMONES**

The very first hypothesis concerning the role of intestinal bacteria in thyroid function appeared in early 1900s (Smith, 1982). Sir Arbuthnot Lane theorized that chronic constipation may lead to systemic dysfunction, including “exophthalmic goitre” (Graves disease) due to toxin absorption from intestine. Basing on this theory D. J. Harries concluded that intestinal bacteria unbalance leads to Graves disease due to an excessive absorption of tryptophan from the intestine, whereas parenchymatous goiter — from an excessive destruction of tryptophan (Harries, 1923). Current studies suggest that sir Lane was not completely wrong, because LPS actually influences thyroid function.

Nonthyroidal illness syndrome (NTIS, euthyroid sick syndrome) is a condition characterized by clinical euthyroidism with low triiodothyronine (T3), total thyroxine (T4), and normal or low thyroid stimulating hormone (TSH) concentration (McIver & Gorman, 1997). NTIS is usually associated with serious disease (either infectious or non-infectious) or wasting. The Gram-negative bacterial LPS exposure may participate in pathogenesis of this condition in several ways. Endotoxin directly and by induced cytokines inhibits hepatic type I iodothyronine deiodinase (D1), that converts T4 to T3 (Yu & Koenig, 2000) and induces the type II iodothyronine deiodinase (D2) in the mediobasal hypothalamus and anterior pituitary gland (Baur et al., 2000; Fekete et al., 2004). Induction of D2, that converts T4 to T3, in the central nervous system may cause suppression of TRH and TSH release. These effects are partly dependent on competition for limiting amounts of coactivators caused by the surplus of cytokines. IL-1, IL-6, TNFs signaling pathways include NF-κB and AP-1, which interact with SRC-1 thus decreasing its availability for other pathways. SRC-1 in healthy individual increases the expression of hepatic D1 gene and its deficit decreases D1 activity (Yu & Koenig, 2000; Boelen et al., 2004). It is worth pointing out that thyroid changes observed after combined administration of IL-1α, TNFα, IL-6 and IFNγ are smaller than after administration of LPS (Boelen et al., 1995). Another mechanism responsible for NTIS during infection is LPS influence on thyroid hormone receptor (TR) in the liver. LPS decreases RXR and TR expression in hepatic extracts, reducing RXR/TR DNA binding (Beigneux et al., 2003).

Na⁺/I⁻ symporter (NIS) plays a crucial role in thyroid physiology by participating in iodide uptake which is the main rate-limiting step in thyroid hormonogenesis. TSH induces NIS expression and stimulates its transport to basolateral membrane of thyrocytes. In turn, iodide inhibits NIS expression and increases NIS protein turnover. In addition, TSH activates NF-κB pathway, which leads to the p65 subunit of NF-κB interaction with a specific xB site at the NIS enhancer (Nicola et al., 2010). Consequently, TSH-induced NIS expression and iodide uptake are stimulated (Nicola et al., 2009). Thyroglobulin (TG) gene expression is also enhanced by LPS, which acts with the involvement of Pax8 and TTF-1 (Vélez et al., 2006). Apart from transcriptional level, LPS modifies TG expression also at posttranscriptional and posttranslational levels.

The exact role of these changes in thyroid hormone metabolism is unclear. Some authors postulate that NTIS should be treated with levothyroxine, despite clinical euthyroidism ( Warner & Beckett, 2010). On the other hand, low T3 concentration may be associated with lower energy expenditure and be beneficial in serious infection. Then, reaction of thyroid gland to bacterial LPS could be an adaptive change and proper physiological reaction to bacterial invasion.

Gut condition is strongly associated with thyroid function (Ebert, 2010). Hypothyroidism may lead to heartburn, dysphagia, vomiting, dyspepsia, intestinal motility disorder and constipation. In turn, hyperthyroidism may be associated with diarrhea. Thyroid hormone’s influence on gut condition is dependent on their direct action in enterocytes. T3 induces intestinal alkaline phosphatase (IAP) and represses lactase gene transcription in these cells, thus regulates their differentiation and function (Meng, 2001). SCFAs produced by the resident microbiota in the intestine lumen may accompany T3 in these processes. SCFAs (butyrate, propionate, acetate) are used by enterocytes as an energy source and are involved in regulation of host appetite and glucose level. They are mainly produced by Clostridium sp., which belong to the Gram-positive phylum of bacteria, Firmicutes. Most of their effects is exerted via free fatty acid receptors (FFAR) 2 and 3 (former G-protein coupled receptors (GPR) 43 and 41), locally in colon (Lin et al., 2012). They stimulate release of incretins, GLP-1 and PYY by intestinal cells L (Tolhurst et al., 2012). Nevertheless SCFAs are absorbed into bloodstream and SCFAs sensing receptors are expressed also in immune cells, adipose tissue and peripheral nervous system (Georgiadi & Kersten, 2012). Main effects of SCFAs on endocrine system seem to be related to energy homeostasis - insulin sensitivity increases after SCFAs treatment, while in adipose tissue the SCFAs improve leptin secretion. These molecules are considered beneficial, improving colon function and carbohydrate metabolism. However, studies performed in vitro suggest that SCFAs may modulate hormonal system function in various locations, such as anterior pituitary gland, where they suppress GH secretion (Ishiwata et al., 2000, 2005) and enhance T3-induced stimulation of prolactin expression (Stanley & Samuels, 1984).

SCFAs inhibit histone deacetylase HDAC-1 activity and activate the Mitogen-Activated Protein Kinase (MAPK) signaling pathway, which leads to hyperacetyl-
atation of some histones and hyperphosphorylation of nuclear receptors (NR) coactivators, which in turn increases transcription of various NR, such as TR (Meng et al., 1999; Jansen et al., 2004). This leads to the enhancement of TRβ-1 function and increases the T3-dependent IAP induction. IAP is a marker of crypt-villus differentiation, so we can conclude that bacterial SCFAs and host T3 cooperate in maintaining the proper intestinal epithelial development and homeostasis (Malo et al., 2013).

Varian et al. showed that L. reuteri supplementation improve function of thyroid gland in mice. L. reuteri consuming mice had higher T4 levels, higher mass of thyroid gland tissue and increased height of the thyroid glandular epithelia as compared with their untreated counterpart (Varian et al., 2014). Mice treated with L. reuteri were also slimmer, more active and had healthier skin than control mice, which correlated with T4 levels. These bacteria induce interleukin-10 production in the intestine, which stimulates host immune tolerance, triggered by anti-inflammatory CD25+ regulatory T cells. In CD25+-depleted mice, L. reuteri supplementation was not beneficial for the thyroid function. Positive effects of bacteria on the thyroid function were also observed in other animals. For instance, female GF rats have smaller thyroids than CV (Vought et al., 1972). Moreover, lactic acid bacteria supplementation in the broiler chicken diets increased the blood plasma thyroid hormone level (Sohail et al., 2014; Chotinski & Mihaylov, 2013). It is suggested that positive impact of probiotics on testicles, thyroid and probably ovaries in animals, inducing changes in G1 microbiome composition may affect both the endocrine and immunological systems, thus influencing aging course (Varian et al., 2014).

Sulfation of T4 and T3 in the liver significantly facilitates deiodination by D1 to inactive derivatives rT3 and T2 (Wu et al., 2005). Under some conditions like propylthiouracil treatment, fetal development, selenium deficiency, or NTIS when D1 activity is low, sulfocjugates may be hydrolyzed to bioactive T4 and T3 due to expression of sulfatases in different tissues and by intestinal bacteria (Kester et al., 2002). However, the significance of gut microflora in maintaining T3 level under conditions of reduced D1 activity is disputed (Veronikis et al., 1996). Physiologically about 20% of serum T3 is of intestinal origin and T3-sulfate (T3S) is a reservoir, which can be recovered by sulfatases. Similarly, major urinary-excreted T3 metabolites — T3 glucuronide — may be hydrolyzed by microflora, which enables EHC of thyroid hormones (de Herder et al., 1989; Mesgarpour et al., 2000). Gut may be an important site in production of bioactive thyroid hormones and intestinal dysbiosis may lead to reduced T3S to T3 conversion and to T3 enterohpatic cycle dysfunction. One study suggests that intestinal bacteria are even capable of deiodination of thyroid hormones (DiStefano et al., 1993). However, this finding remains unconfirmed.

7α-dehydroxylation of primary bile acids by intestinal microbiota results in formation of secondary bile acids: lithocholic and deoxycholic. Main 7α-dehydroxylation species isolated from human feces include Clostridium and Escherichia (Wells & Hylemon, 2000). Taurine-conjugated secondary bile acids, taurolithocholic acid and taurodeoxycholic acid are the most potent TGR5 ligands. In response to these compounds, the G-protein-coupled receptor TGR5 activates and subsequently intracellular cAMP level raises. Afterward, in brown adipocytes and human skeletal myocytes induction of the cAMP-dependent D2 occurs, which promotes energy expenditure (Watanabe et al., 2006). Paradoxically, some studies show that GF rats have significantly higher T3 to T4 ratio in the blood plasma than conventional animals (Ukaï & Mitsuma, 1978). It is possible that greater reabsorption of bile acids in these animals may lead to enhanced T3 producing enzymes expression (Wostmann, 1973).

GROWTH HORMONE

GH is secreted by anterior pituitary gland in a pulsatile manner dependent on the action of GH releasing hormone (GHRH) and ghrelin, which stimulates GH secretion, and somatostatin which inhibits GH secretion (Vijayakumar et al., 2010). The physiological effects of GH results from direct by interacting with a specific receptor on the surface of cells and stimulation of insulin growth factor 1 and 2 (IGF-1 and -2) secretion by liver. Lactobacillus plantarum may promote Drosophila systemic growth by affecting the TOR-dependent host nutrient sensing system controlling hormonal growth signaling (Storelli et al., 2011). Similarly, L. rhamnosus administration to zebrafish cause elevation of IGF-1, IGF-II, thus accelerates backbone calcification (Avella et al., 2012). It has been hypothesized that intestinal microbiota may also influence growth of mammals. For instance, in rabbits fed with probiotics, GH level elevates (Ghoneim & Moselhy, 2013), whereas human infants fed with formula enriched with L. rhamnosus GG grew better compared to their control counterpart (Vend et al., 2006).

LPS is a known exogenous factor which affects GH secretion and action (Figure 2). Studies performed on rats and domestic fowls revealed that LPS causes GH release inhibition (Curtis et al., 1980; Kasting & Martin, 1982), whereas in human and sheep transitionally enhances GH secretion, probably acting on pituitary level (Lang et al., 1997; Briard et al., 1998; Daniel et al., 2002). Despite sustained increase in GH concentration, LPS injection results in a state of resistance to GH. LPS increases the production of proinflammatory cytokines like TNF-α, interleukin-1β (IL-1b), and interleukin-6 (IL-6). TNF-α suppresses GH receptor (GHR) expression by reduction of transactivators Sp1/Sp3 binding to a GHR promoter (Denson et al., 2001), IL-6 up-regulates strong inhibitor of GH intracellular signal transduction, SOCS-3 (Wang et al., 2002a; Denson et al., 2003). Other mechanisms of LPS-dependent GH resistance are: decrease in IGF-1 concentration caused by liver cyclooxygenase-2 activation (Briard et al., 2000; Wang et al., 2002b; Martin et al., 2008), direct suppression of GHR expression by MyD88-dependent and -independent TLR4 signaling pathways (Dejkmarn et al., 2007) and promotion of proteolytic GHR cleavage (Wang et al., 2008). Moreover, the ability of GH to promote phosphorylation of signal transducer and activator of transcription (STAT) in the liver is reduced during sepsis (Hong-Brown et al., 2003). Furthermore, LPS administration alters IGFBP serum concentrations. In normal condition, IGFBP-3 forms a stable ternary complex with IGF and the acid labile subunit (ALS), so that IGF half-life is extended, IGFBP-1 serves as an inhibitor of IGF bioactivity, whereas IGFBP-2 is an optional IGF carrier in condition of low IGFBP-3 level (Donaghy & Baxter, 1996). LPS administration promotes increased IGFBP-3 proteolysis in serum and/or its decreased synthesis in the liver (Priege et al., 2003), while IGFBP-1 accumulates in certain tissues, like skeletal muscles and blocks IGF-dependent protein synthesis (Frost & Lang, 2004).

These studies may be crucial to understanding the mechanism of growth failure in children with inflamma-
tory bowel diseases (IBD). Due to increased intestinal permeability, systemic endotoxemia is a common finding in these patients and may underpin extra-intestinal complications. Chronic elevation of LPS binding protein (LBP) serum level has been proposed as a potentially useful marker of high refractory growth failure risk in pediatric IBD (Pasternak et al., 2010). During chronic infection or after sepsis, GH resistance develops in peripheral tissues, that may result for instance in muscle mass loss. Thus, LPS-dependent pathways leading to GH resistance may become the future therapy targets.

Another mechanism of bacteria-associated growth failure in children is observed in Helicobacter pylori carriers. An assessment of the anti-H. pylori antibodies in GH deficient short stature group, an idiopathic short stature group and control group, revealed that in children with idiopathic short stature the antibody positivity rate was significantly higher than in other groups (Takahashi et al., 2002). Possible explanation of this effect is decreased ghrelin production by enteroendocrine cells in the gastric mucosa. Ghrelin is a GH releasing peptide as a GH secretagogue type 1A receptor (GHSR) ligand and has an important role in maintaining energy homeostasis and regulation of hunger. Chronic damage of gastric ghrelin-positive cells during H. pylori infection leads to decreased GH secretion, impaired hunger signals and in consequence growth retardation. Importantly, body composition improves in children after therapy (Osawa et al., 2006). Surprisingly, in some patients after H. pylori eradication, plasma ghrelin concentrations decrease and BMI increases (Pacifico et al., 2008). These variances may be dependent on the strain of bacteria: type I (producing the cag) is associated with lower ghrelin concentration than type II (without virulence factors), so eradication of different strains may lead to different effects (Deng et al., 2012).

Maternal oral Campylobacter rectus or P. gingivalis infection may cause intrauterine growth restriction (IUGR). These bacteria may invade placental tissues and promote hypermethylation in the promoter region-P0 of the Igf2 gene (Bobetsis et al., 2007). TLR-4 knockout mice do not develop IUGR phenotypes after systemic C. rectus infection, which emphasizes the crucial role of this receptor in placental immunity (Arce et al., 2012).

**PARATHYROID HORMONE AND GUT-KIDNEY AXIS**

Due to the deterioration of renal clearance during chronic kidney disease (CKD), many compounds accumulate and contribute to the uremic syndrome. These

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**Figure 2. LPS influence on GH action.**

Details, see main text. COX-2, cyclooxygenase-2; IGF-1, insulin like growth factor 1; IGFBP3, IGF binding protein 3; GH, growth hormone; GHR, GH receptor; LPS, lipopolysaccharide.
molecules, called uremic toxins are classified as: small water-soluble compounds (urea, guanidines, oxalate, phosphorus, polyamines), protein-bound compounds (p-cresol and p-cresylsulfate, indoles, homocysteine, furan-propanic acid) and middle molecules (β2-microglobulin, PTH) (Duranton et al., 2012). The originator of some uremic toxin is colon microbiome, which changes its composition during CKD. Low dietary fiber intake and impaired protein assimilation in the small intestine decreases carbohydrate to protein ratio in colon, thus promoting proteolytic bacterial species over saccharolytic ones. SCFA production by Lactobacillaceae and Prevotellaceae families decreases, whereas Bacteroides family overgrows and generates toxic solutes (Poesen et al., 2013). Gut bacteria convert tryptophan to indole, which is hydroxylated and sulfonated to indoxyl sulfate (IS) in the liver, whereas p-cresyl sulfate (PCS) is the product of bacterial tyrosine degradation followed by sulfate conjugation. Bone mineralization deficiency in CKD is called renal osteodystrophy. Endocrine changes which cause this condition are secondary hyperparathyroidism (SHP) and low vitamin D activation rate in kidneys. SHP in CKD may be a result of phosphate retention, hypocalcemia, decreased production of or resistance to calcitriol, abnormal sensitivity to calcium, direct effects of phosphate and skeletal resistance to PTH (Fukagawa et al., 2002). The mechanism of the latter stimulus, skeletal resistance to PTH, is related to IS and PCS produced by anaerobic bacteria. IS is taken up by osteoblasts via organic anion transporter 3 (OAT3) and afterward increases intracellular free radical production. Furthermore, IS down-regulates PTHR gene and decreases PTH-induced cAMP production in osteoblasts, that might lead to skeletal resistance to PTH (Nii-Kono et al., 2007; Goto et al., 2010).

Uremic toxins produced by bacteria might have an impact on function of other hormones. For instance, PCS activates ERK1/2 in mice skeletal muscle, thus promoting insulin resistance (Koppe et al., 2013). Also, uremic patients usually have low total T3 level, which may be caused by inhibition of T4 hepatocyte transport and subsequent T4 deiodination by IS (Lim et al., 1993).

CONCLUSIONS

Bacterial influence on endocrine system is difficult to understand and investigate, however new metabolomics and bioinformatics research provide information about bidirectional cross-talk between host hormones and microbiome. This knowledge is necessary to comprehend hormonal changes during a bacterial infection, antibiotic or probiotic treatment and diet modifications. Rational modifications of intestinal flora may decrease some hormone-dependent diseases risk, like breast and prostate cancer.

REFERENCES


