



hCG, the Centerpiece of Life and Death

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ARTICLE INFO

Article type:
Review Article

Article history:
Received: 05 Feb 2011
Revised: 30 Feb 2011
Accepted: 20 Mar 2011

Keywords:
Human Chorionic Gonadotropin
Cytotrophoblast
Choriocarcinoma

ABSTRACT

hCG is a generic name for 5 biologically active molecules that share a common α and β -subunit amino acid sequence. These 5 molecules have key biological function in human pregnancy and human cancer. This review examines these molecule in detail. These 5 molecules, hCG, sulfated hCG, hyperglycosylated hCG, hCG free β and hyperglycosylated free β are produced by placental syncytiotrophoblast cells and pituitary gonadotrope cells (group 1), and by placental cytotrophoblast cells and human malignancies (group 2). Group 1 molecules are both hormones that act on the hCG/LH receptor. These molecules are central to human menstrual cycle and human pregnancy. Group 2 molecules are autocrines that act by antagonizing a TGF β receptor. These molecules are critical to all advanced malignancies. The hCG groups are molecules critical to both the molecules of pregnancy or human life, and to the advancement of cancer, or human death.

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► Implication for health policy/practice/research/medical education:

Article shows the multiple functions that hCG variant have in pregnancy, and in normal pituitary function in women. As also shown, hCG variants are misused by all human malignancies to drive growth, invasion and metastases.

► Please cite this paper as:

Cole LA. hCG, the Centerpiece of Life and Death. *Int J Endocrinol Metab.* 2011;9(2):335-52. DOI: 10.5812/kowsar.1726913X.3398

1. hCG, Two Groups of Molecules With Common Amino Acid Sequences

It is not easy say who was the discoverer of the placental gonadotropin hCG. In 1912, Aschner stimulated guinea pig genital tracts with injections of extracts of human placenta (1). In 1919, Hirose used immature rabbits and human placental tissue to stimulate ovulation and normal luteal function (2). Both of these studies show a clear link between a hormone produced by the placenta and the uterus. Around this time, the name human chorionic gonadotropin (hCG) was conceived: Chorion comes from latin "chordate" meaning after birth; gonadotropin because the hormone is a gonad-tropic molecule, acting on the ovaries, promoting steroid-induced actions. In 1927, Ascheim and Zondek demonstrated that pregnant

women produced a gonad-stimulating substance (3). This was in some respects the first finding of hCG. They showed that injecting this molecule into female mice led to follicular maturation and ovulation. We claim in *Table 1* all of these authors, Aschner, Hirose, Ascheim and Zondek 1912-1927 as the discoverers of hCG. The review is the incredible story of molecule hCG as we know it today.

Table 1 shows major discoveries in the story of hCG (1-36). Numerous studies focused on the amino acid sequence of hCG (27), and the N- and O-linked oligosaccharides that form a major part of the hCG structure (14, 26). Other major discoveries include the hCG/LH receptor (7, 25) and the unraveling of the many biological activities of hCG (24). Possible the intriguing function found for hCG is that it controls growth if the fetus (see Section 2, hCG and pregnancy). Numerous investigators have unraveled the story showing that cancer cells produce an hCG form. This research starts with the finding that cancers produce hCG free β (17). It goes on to the discovery that all advanced cancers produce hCG free β (4). As discovered in 2000, hCG free β promotes cancer cell growth

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and malignancy by antagonizing a TGF β receptor, a different receptor to the hCG/LH receptor. Other major discoveries include the finding of a common α -subunit gene on chromosome 6 (20), of a β -subunit gene on chromosome 19 (12), and of the hCG β -LH β complex genes on chromosome 19 (29) (Table 1). As described, the first hCG test, a biological activity test, was discovered in 1930 (36), and the original hCG antibody agglutination test was discovered in 1960 (35). The initial hCG radioimmunoassay was discovered in 1967 (6). The initial radioimmunoassay cross-reacted with LH (6). Vaitikaitis and colleagues developed the hCG β radioimmunoassay, which differed hCG from LH (33). Free α -subunit and free β -subunit were discovered as secreted molecules in 1978 (21). The carbohydrate structure that prevents free α -subunit combination with β -subunit was described in 1985 (10). Seemingly, the oddest discovery was that some hCG variants function independent to the hormone hCG (9, 13, 15, 16). As shown, hyperglycosylated hCG promotes cytotrophoblast cell growth during pregnancy, and drives pregnancy implantation/ This molecule has independent functions to hCG (16, 37-40). As recently demonstrated, hyperglycosylated hCG functions as pregnancy implantation promoter and hCG free β functions as a cancer promoter, both function by antagonizing a TGF β receptor, rather than acting on the hCG/LH receptor (13, 15). Multiple forms of hCG include sulfated forms of hCG that is produced by the pituitary gland, during the menstrual cycle (9, 31). These sulfated hCG molecules are hormones like hCG, acting on the LH/hCG receptor. Sulfated hCG supplements LH during the menstrual cycle (31).

As shown in Table 2. there are 5 clear variants of hCG, produced by different cells and having separate functions. These are all molecules that share a common amino acid sequence with hCG. I remember that with the discovery of the first independent variant, hyperglycosylated hCG, produced by different cells and having separate functions (16), I initially gave it a separate name, Invasive Trophoblast Antigen. This name was used for 2 years, until World Health Organization (WHO) informed me that it had to have a name that included the term hCG, since it had an identical amino acid sequence to hCG. This is such a strange situation, hCG binds the LH/hCG receptor and hyperglycosylated hCG antagonizes a TGF β receptor. Two separate molecules with a common amino acid sequence. I promptly renamed it hyperglycosylated hCG. I assume the same rule must apply to all 5 hCG variants. The hCG variants all vary in meric structure (number of subunits) and carbohydrate structure (Table 2). hCG is the most glycosylated glycoprotein known. The hCG dimer comprises 30% sugars or oligosaccharides by molecular weight, hyperglycosylated hCG comprises 39% oligosaccharides, and hyperglycosylated free β comprises 42% oligosaccharides by molecular weight. Of the 5 biologically active variants, hCG is made by placental syncytiotro-

phoblast cells, sulfated hCG made by pituitary gonadotrope cells, hyperglycosylated hCG made by placental cytotrophoblast cells, hCG free β made by malignancies and hyperglycosylated free β made by malignancies. These 5 variants can be classed into two general types of molecules. hCG and sulfated hCG are endocrines, acting on an hCG/LH receptor during pregnancy (hCG) and during the menstrual cycle (sulfated hCG) (9, 31, 41). Hyperglycosylated hCG, hCG free β and hyperglycosylated free β , in contrast, are all autocrines, acting on the TGF β receptor on the cells that produce the molecules (13, 16, 37). So there are the hCG endocrines and the hCG autocrines. The autocrines are all cancer promoters (15).

hCG is composed of a 92 amino acid α -subunit with 2 N-linked oligosaccharides, and a non-covalently linked 145 amino acid β -subunit with 2 N-linked oligosaccharides and 4 O-linked oligosaccharides (27, 42, 43). The structure of each variant of hCG varies considerable in carbohydrate structure (42, 43). The structures produced in cancer cases, choriocarcinoma and other malignancies include larger hyperglycosylated structures, with triantennary N-linked oligosaccharides and more complex O-linked oligosaccharides (14, 26, 42, 43). hCG molecules also vary greatly in charge (44), due to variation in sialic acid sugar content. As if that is not enough heterogeneity there is another major source for hCG heterogeneity. hCG is nicked and dissociated immediately after secretion by macrophages, leukocytes and monocytes (45). These accumulate around placental trophoblast cells. The enzyme human leukocyte elastase is produced by macrophages, leukocytes and monocytes. It cleaves hCG-related molecules at β 45-46 or β 47-48 (45). This same enzyme then cleaves the hCG β -subunit C-terminal peptide at β 92-93 leading to the release of the β -subunit C-terminal peptide (5, 46, 47). Add to this hCG dissociation, and the more rapid dissociation following nicking by leukocyte elastase (45) and one has the major pathways of hCG degradation. In kidneys, following nicking or cleavage of the β -subunit C-terminal peptide in serum, exoglycosidases clear β 39-47 and β 48-55, leukocyte elastase, the nicking enzyme, cleaves β 5-6. What appears in urine is β -core fragment, the terminal degradation product of hCG variants. β -core fragment comprises β -subunit 6-38, disulfide linked to β 55- β 92.

Adding everything together, hCG exist as 5 independent biologically active molecules. Each of these may be synthesized as fully sialylated, incompletely sialylated, partially sialylated and minimally sialylated variants. Five molecules times 4 extents of sialylation is 20 molecules. Once secreted, each of these may be nicked or not nicked at β 44-45 or β 47-48, taking us from 20 molecules to 60 molecules. These may remain intact in the circulation or dissociated as free β -subunit, bringing us to 120 circulating, molecules. These may be cleaved and loose their C-terminal peptide bring us up to a mixture of 240 circulating molecules. When one considers immunoassays for detecting total hCG in serum. What an impossi-

Table 1. Major Discoveries in the hCG Field (1912 to Present).

| | | |
|---|---|-----------|
| Aschner, Hirose, Ascheim and Zondek (1-3) | First discovery of placental ovarian gonadotropin, hCG | 1912-1927 |
| Zondek B, Ascheim S (36) | First hCG test, the Zondek-Ascheim Pregnancy Test | 1930 |
| Wide L, Gemzell CA (35) | First immunological pregnancy test, an antibody agglutination test | 1960 |
| Aono T, et al (6) | First hCG radioimmunoassay | 1967 |
| Swaminathan N, et al (32) | Discovery that hCG is a non-covalently linked dimer | 1970 |
| Vaitukaitis JL, et al (33) | Discovery of hCG β radioimmunoassay, assay only detects hCG | 1972 |
| Morgan FJ, et al (27) | Discovery of the amino acid structure of hCG | 1975 |
| Franchimont P, et al (21) | Production of hCG free α -subunit and free β -subunit | 1978 |
| Fiddes JC, et al (100) | Discovery of hCG evolution among primates | 1980 |
| Wehmann RE, et al (34) | Discovery of hCG metabolic clearance rate | 1981 |
| Fiddes JC, et al (20) | Discovery of common α -subunit gene | 1981 |
| Boorstein W, et al (12) | hCG β -subunit gene structure | 1982 |
| Mizouchi T, et al (26) | Discovery of the N-linked carbohydrate structure of hCG | 1983 |
| Cole LA, Hartle Ret al (17) | Discovery that free β subunit detected in cancer cell lines, and detected in cancer patient serum and urine | 1983 |
| Hussa RO, et al (22) | First hCG immunometric assay | 1984 |
| Amr CE, et al (5) | Discovery of β -core fragment and of free hCG β C-terminal peptide, hCG breakdown products in urine | 1984 |
| Blithe DL, et al (10) | alternative structure of hCG free α -subunit, why does not combine with β | 1985 |
| Policastro PF, et al (29) | Map of hCG and LH genes | 1986 |
| Bogart MH, et al (11) | hCG as a marker of Down syndrome pregnancy | 1987 |
| Odell WD, et al (31) | Demonstration that pituitary gland produced hCG during menstrual cycle | 1987 |
| Cole LA (14) | Discovery of the O-linked carbohydrate structure of hCG | 1987 |
| Nishimura R, et al (28) | Discovery of Nicked hCG | 1988 |
| Ascoli M, et al (7) | Discovery hCG/LH receptor and its mode of action | 1989 |
| McFarland RC et al. (25) | Discovery hCG/LH receptor and its mode of action | 1989 |
| Lei ZM, et al (24) | Discovery of multiple biological functions for hCG | 1992 |
| Laphorn AJ, et al (23) | Discovery of hCG 3D structure, demonstration that it contains common structures with TGF β , PDGF and NFG | 1994 |
| Acevedo HF, et al (4) | Discovery that all cancers produce an hCG-related molecule | 1996 |
| Birken S, et al (9) | Discovery of sulfated hCG produced by pituitary with reduced bioactivity | 1996 |
| O'Connor JF, et al (30) | Discovery that hyperglycosylated hCG predominates in early pregnancy | 1998 |
| Butler SA, Iet al (13) | Discovery that cancer cell hCG free β acts separately to hCG, antagonizing cancer cell TGF β receptor | 2000 |
| Birken S, et al (8) | Establishment of pure hCG standards | 2003 |
| Cole L, et al (16) | Discovery of hyperglycosylated hCG as an independent molecule to hCG | 2006 |
| Cole LA, et al (18) | Discovery of the role of hCG in human evolution | 2008 |
| Cole LA, et al (15) | That hyperglycosylated hCG, hCG free β and hyperglycosylated free β are interchangeable cancer promoters, all antagonizing a TGF β receptor | 2011 |

ble job, appropriately detecting 240 different variants of hCG.

Most hCG-related molecules are cleared from the circulation by liver. As estimated, 78% of hCG-related molecules are cleared by the human liver (48). Molecules deficient in sialic acid sugar residues are cleared through the liver galactose receptor (49). All told the liver and

the renal urinary β -core fragment clearance system clear all hCG-related molecules

hCG enzyme immunometric assays include the 12 automated platform assays used by every clinical laboratory all over the world (Abbott AxSYM system, Abbott Architect system, Beckman Access system, Beckman Dxl 800 system, Ortho Vitros ECIQ system, Roche Elecsys

Table 2. Properties of the Independent Variants of hCG.

| | hCG | Sulfated hCG | Hyperglycosylated hCG | hCGβ | Hyperglycosylated hCGβ |
|--|---------------------|---------------------|------------------------------|------------------------------|--|
| Source cell | Syncytiotrophoblast | Gonadotrope | Cytotrophoblast | Advanced malignancy | Advanced malignancy |
| Mode of action | Endocrine | Endocrine | Autocrine | Autocrine | Autocrine |
| Type of molecule | hCG Endocrine | hCG Endocrine | hCG autocrine | hCG autocrine | hCG autocrine |
| Total molecular weight | 37,180 | 36,150 | 42,800 | 23,300 | 27,600 |
| Site of action | LH/hCG receptor | LH/hCG receptor | TGF β antagonism | TGF β antagonism | TGF β antagonism |
| Amino acids α -subunit | 92 | 92 | 92 | - | - |
| Amino acids β -subunit | 145 | 145 | 145 | 145 | 145 |
| Peptide molecular weight | 26,200 | 26,200 | 26,200 | 16,000 | 16,000 |
| O-linked oligosaccharides | 4 | 4 | 4 | 4 | 4 |
| N-linked oligosaccharides | 4 | 4 | 4 | 4 | 4 |
| Molecular weight sugars | 10,980 | 9,950 | 16,600 | 7,300 | 11,600 |
| Sugars, % | 30 | 28 | 39 | 31 | 42 |
| Isoelectric point (pI), principal peak | 3.5 | Not known | 3.2 | Not known | 3.5 |
| Metabolic clearance rate, h | 36 | 20 | Not known | 0.72 | Not Known |

Amino acid content, molecular weight and sugar contents determined from published structures as determined by Elliott, *et al.* for hCG and hyperglycosylated hCG (42), Birken *et al.* for Sulfated Pituitary hCG (9) and Valmu *et al.* for Hyperglycosylated hCG β (43).

The molecular weight of common hCG dimer amino acid backbone is that as determined by Morgan *et al.* (27). Molecular weight of N- and O-linked sugar side chains is added to these values. Isoelectric points are those published by Sutton *et al.* (44) and metabolic clearance rate are those established by Wehmann and Nisula (34). Modes of action are sites of action are as published (8, 9, 13, 15, 41).

system, Siemens ACS180 system, Siemens Centaur, Siemens Dimension Vista system, Siemens Immulite system, Siemens Status system and Tosoh AIA system). It is amazing, how these automated assays detect these extremes of heterogeneity with the existence of as many as 240 hCG variants in human blood. The answer is that they do not, and this confuses serum total hCG results accordingly. In general (50, 51), all assays appropriately detect intact hCG, but invariably detect or do not detect nicked hCG, hyperglycosylated hCG, nicked hCG missing the C-terminal peptide, hCG free β -subunit, nicked hCG free β -subunit, β -core fragment, and hCG deficient in or variable in sialic acid. The only exception that we are aware of is the hCG Immulite systems (Immulite 1000, 2000, 2500) total hCG assay, and the old hCG β radioimmunoassay. These are only assays that detect all of these major hCG variants (50, 51).

In conclusion, hCG is an extremely heterogeneous molecule. This review examines these molecules in detail. While hCG is clearly the key hormone in pregnancy, variants such as sulfated hCG are key to pituitary functions. hCG free β and hyperglycosylated are seeming key promoters in human cancer biology.

2. hCG and Hyperglycosylated hCG, the Potent Growth, Angiogenic and Invasion Factors That Drive Human Pregnancy

hCG and hyperglycosylated hCG are the endocrine and the autocrine which control pregnancy. Of the 5 hCG variants described in Table 2, just these two drive multiple aspects of pregnancy. Pregnancy, of course, is always detected by a total hCG test, with the initial detection of primarily hyperglycosylated hCG) or the much less concentration of hCG) on the day of implantation (52-54). Figure 1 shows the logarithmic levels of the hormone hCG and the autocrine hyperglycosylated hCG produced during pregnancy (52-54). The initial placental cells or root cells are mononuclear cytotrophoblast cells. The cell produce hyperglycosylated hCG. These cells fuse forming differentiated polynuclear syncytiotrophoblast cells. These differentiated cells produce hCG. New placenta is being generated throughout the course of pregnancy so that new cytotrophoblast cell are produced making hyperglycosylated hCG and then fused to make cells that produce hCG. As a result, hCG and hyperglycosylated hCG are produced through the length of pregnancy (Figure 1). The new placenta that

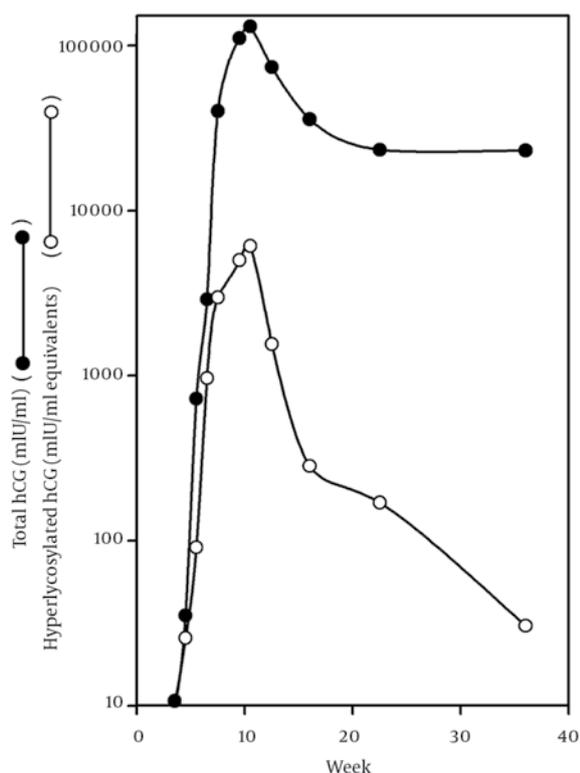


Figure 1. Serum Median Concentration of Total hCG (●) and Hyperglycosylated hCG (○) Produced During Pregnancy (n=536) [52-54].

is implanted comprises of mostly root cytotrophoblast cells. As such, hyperglycosylated hCG comprise 90% of serum total hCG in the 3rd week of gestation or the week following implantation of pregnancy. Hyperglycosylated hCG comprises 73% of total hCG at 4 weeks, 50% of total hCG at 6 weeks dropping down to 10% hyperglycosylated hCG at 10 weeks gestation and 1/2% hyperglycosylated hCG in the third trimester of pregnancy (52-54). Both hCG and hyperglycosylated hCG peak at around 10 weeks of gestation. At peak, total hCG concentration is close to 100,000 mIU/ml or almost 40,000-fold greater than mean implantation hCG levels. hCG is the only know molecule to be stimulated so greatly. Hyperglycosylated hCG levels at peak are approximately 8,000 mIU/ml equivalents, or just 1,500-fold amplified over implantation concentration. Following the peak, hCG and hyperglycosylated hCG concentrations drop significantly. hCG and hyperglycosylated hCG levels can vary very greatly between individual pregnancies. At 4 weeks gestation, total hCG levels in term outcome pregnancies can vary from 2.0 to 1760 mIU/ml or vary by 888-fold, at 5 weeks gestation total hCG levels can vary from 11 to 7680 mIU/ml or vary by 700 fold, and at 6 weeks of gestation can vary from 8.4 to 6920 mIU/ml or vary by 820-fold (55). How can normal term outcome hCG concentration vary so greatly? As demonstrated, variation comes from inappropriately dating pregnancies, dating them to the start of the last

menstrual period rather than to day of implantation (start of appearance of low concentrations of hCG) (55). The other issue that cause the wide variation in hCG levels is that individual pregnancies amplify hCG differently. In some pregnancies cells amplify total hCG in early pregnancy as slow as 1.52-fold per day over multiple days, while in other pregnancies hCG may be amplified by as much as 2.92-fold over multiple days (53). It appears that both of these factors, together cause the wide variation in individual hCG production in normal term pregnancy (53). A separate major factor copes with making sure all pregnancies are normal and that hCG critical biological activities can cope with these wide variations. This is the spare receptor theory (56-58). Under the spare receptor theory, when a small proportion of receptors is activated it may yield similar cellular response to when all receptors are activated (56-58). This is due to plateaus in receptor G protein and cyclic AMP response (56-58). Similarly, the low progesterone production produced in response to high concentrations of hCG may be explained by down-regulation (59, 60). As demonstrated, high concentrations of hCG decrease the number of receptor on cells by degrading the receptor transcript rate. A significant decrease has been observed in half-life of receptor transcript (59). This may explain the low progesterone production in pregnant women with high total hCG levels. Together, the spare receptor and down-regulation theories explain how the hCG/LH receptor copes with the extreme of total hCG concentrations. The 3 dimensional structure of hCG is shown in Figure 2 (23). As shown, the 145 amino acid β -subunit folds itself around the 92 amino acid α -subunit in a non-covalent manner. A clear

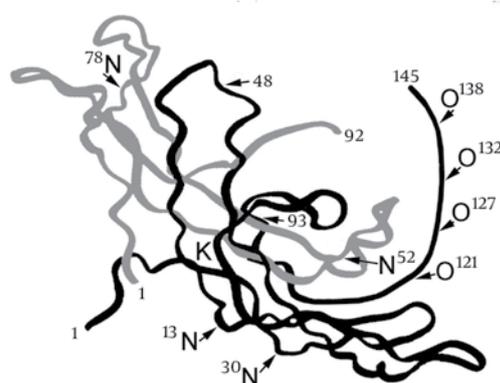
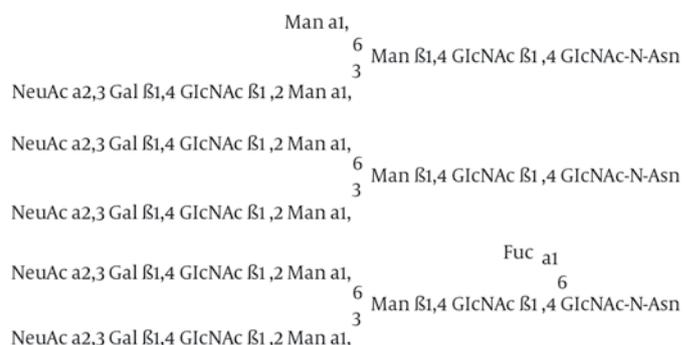


Figure 2. Crystal Structure of Deglycosylated Regular hCG, as Shown by Laptorn, et al. (23).

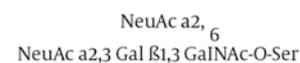
The unfolded β -subunit C-terminal peptide is added (missing in crystal structure). It is inferred that this structure is not folded since the sequence comprises primarily a polymer of proline and serine residues. The symbol N indicate the site of attachment of N-linked oligosaccharides, and the symbol O the attachment site of O-linked oligosaccharides. Residue β 48 is indicated, the site of nicking of hCG (β 47-48), and residue β 93, the site of cleavage of the β -subunit C-terminal peptide (β 92-93). The symbol K indicates the site of the cystine knot structure, 4 peptides β 1-15, β 30-45, β 80-100, and β 50-65 linked by 3 disulfide bridges, β 34-88, β 9-57 and β 38-90. The α -subunit is shown in grey, and β -subunit in black.

N-linked oligosaccharides

hCG and hCG β



O-linked oligosaccharides



Hyperglycosylated hCG and hyperglycosylated hCG β

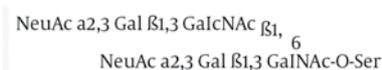
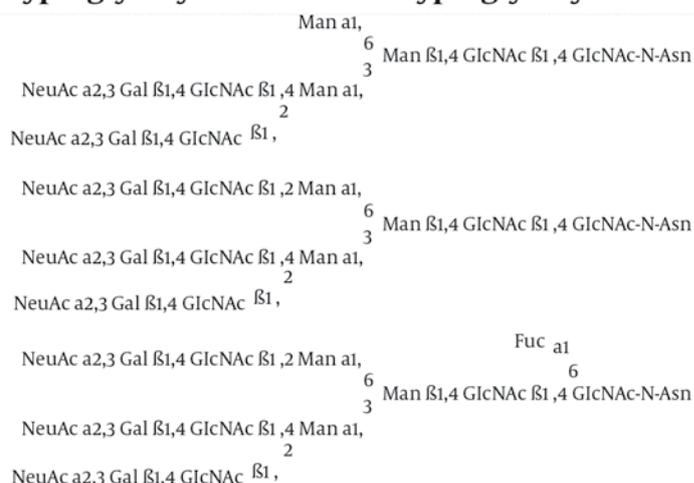


Figure 3. Structure of N-linked Oligosaccharides and O-linked Oligosaccharides Attached to hCG (and hCG Free β), and Hyperglycosylated hCG.

structural arm of β -subunit, residues 60-95 literally folds around α -subunit, like it cuddles or hold on to this subunit. The crystal structure had to be determined on deglycosylated hCG. Figure 3 shows the structure of sugars or oligosaccharides attached to hCG (42, 43). As shown, hCG has primarily monoantennary and biantennary N-linked oligosaccharides, and trisaccharide and tetrasaccharide O-linked oligosaccharides.

hCG and hyperglycosylated hCG drive almost every aspect of pregnancy. The big exception is balancing nutrients during pregnancy, human placenta lactogen (hPL) and human placental growth hormone (hPGH), two growth hormone analogs, fill this critical function, balancing glucose supply and amino acid supply reaching the fetus in a pregnant woman. For some reason, all reproductive biology and obstetrics and gynecology text books sold today claim that maintaining the corpus luteum during early pregnancy is the sole job of hCG. This is grossly incorrect, over 200 solid publications show and confirm and confirm again that hCG and hyperglycosylated hCG performs a very large number of key roles during pregnancy (1-3, 16, 24, 37-40, 61-98). As shown in Table

3, hCG and hyperglycosylated hCG control many aspects of pregnancy (1-3, 16, 24, 37-40, 61-98). hCG maintains corpus luteal progesterone production during pregnancy. hCG and hyperglycosylated hCG together control establishment of ultra-efficient hemochorial placentation. hCG and hyperglycosylated hCG control villous placentation, and hCG promotes uterine spiral blood vessels, and generates umbilical blood vessels, to meet the villous placentation structures. hCG controls uterine growth during pregnancy and suppresses immunorejection of the foreign fetoplacental unit. hCG suppresses contractions during pregnancy, and hyperglycosylated hCG controls implantation of pregnancy. Most of all, multiple researchers have identified hCG receptors on fetal organs, and have located a fetal source of hCG production. It is indicated that hCG is the key fetal growth factor during pregnancy. All told, hCG is the critical pregnancy factor (1-3, 16, 24, 37-40, 61-98). Starting at implantation of pregnancy, hCG takes over from LH maintenance of corpus luteal progesterone production (1, 2, 63, 75, 78, 81, 82, 86, 91, 95). This continues for just 3-4 weeks in pregnancy. With time, placenta matures with fusion and differen-

Table 3. The Biological Functions of hCG and Hyperglycosylated hCG. References Cited From 44 of Over 200 Published Articles.

| Signal Molecule | Function | References |
|-----------------------------|--|---|
| hCG | 1. Promotion of corpus luteum progesterone production | (1-3, 63, 75, 78, 81, 82, 86, 91, 95) |
| | 2. Angiogenesis of uterine vasculature | (24, 64, 74, 92, 93, 97, 98) |
| | 3. Cytotrophoblast differentiation | (90) |
| | 4. Immuno-blanding and blockage of phagocytosis of invading trophoblast cells | (62, 67, 77) |
| | 5. Growth of uterus in line with fetal growth | (89, 96) |
| | 6. Quiescence of uterine muscle contraction | (66, 69, 70, 89) |
| | 7. Promotion of growth and differentiation of fetal organs | (60, 61, 72, 79, 89) |
| | 8. Blastocysts signal decidua prior to implantation | (80, 84, 89) |
| | 9. Umbilical cord growth and development | (87, 88, 94) |
| | 10. hCG in sperm and receptors found in fallopian tubes suggesting pre-pregnancy communication | (68, 71, 76) |
| | 11. hCG receptors in brain hippocampus, hypothalamus and brain stem, cause of pregnancy nausea/vomiting | (76, 79, 83, 85) |
| Hyperglycosylated hCG | 1. Blocks apoptosis in cytotrophoblast cells | (73) |
| | 2. Promotes invasion process by cytotrophoblast cells | (16, 37-39) |
| | 3. Promotes implantation of pregnancy | (16, 37-40) |
| | 4. Promotes growth of cytotrophoblast cells | (16, 37-40) |
| hCG + hyperglycosylated hCG | Together promotes cytotrophoblast growth and fusion to form villous placenta. This then fuses with promoted uterine circulation and promoted umbilical circulation to initiate hemochorial placentation. | (15, 16, 37, 64, 65, 68, 74, 87, 88, 90, 92-94, 97, 98) |

tiation becoming villous placental tissue. This then takes over production of progesterone. Clean transfers occur without a nick in progesterone levels from rising corpus luteal LH-promoted progesterone to rising hCG-promoted progesterone with implantation, and then rising syncytiotrophoblast progesterone after the 6th-7th week of gestation.

One of the key jobs the hormone hCG in pregnancy, joint with hyperglycosylated hCG, is establishment of the ultra-efficient fetal nutrient exchange or hemochorial placentation. The reason why it is called ultra-efficient are explained under Section 3, hCG and Evolution (18). This explains how hemochorial placentation has to be increased in primates to the highest possible efficiency to accommodate human brain development. As shown in *Figures 4 and 5* villous placenta is first generated. At the same times as hCG promotes villous formation it promotes uterine angiogenesis and formation of the umbilical circulation. These all meet to initiate hemochorial placentation. *Figure 4* Panel A shows cytotrophoblast columns in conceptus promoted by hyperglycosylated hCG (15, 16, 37-40). *Figure 4* Panels B and C shows columns of cytotrophoblast cell protruding from this tissue. The growing column become differentiated around the edges promoted by hCG, to form syncytiotrophoblast tissue (90). As a result of this process villous structures are formed, see *Figure 4* Panels C and D. Once villous structures are formed they combine with growing umbilical and maternal circulations to initiate hemochorial placentation. hCG promotes growth or angiogenesis of uterine arteries to

meet this growing villous tissue (24, 64, 74, 92, 93, 97, 98). hCG also controls the growth of umbilical vasculature to meet this developing hemochorial placentation. *Figure 5* illustrates active hemochorial placentation, where the villous trophoblast meets with maternal spiral arteries and a umbilical circulation. This process comprises 3 of hCG's prime functions (Functions 2,3 and 9 in *Table 3*) and 1 of hyperglycosylated hCG prime functions. Other critical hCG functions include the immuno-blanding of the fetoplacental unit and blockage of phagocytosis by macrophages (62, 67, 77). hCG makes sure that the uterus grows and does not stretch, in line with fetal growth (89, 96). hCG also inhibits myometrial muscle contractions during the course of pregnancy (66, 69, 70, 89). hCG levels drop to their lowest concentration at parturition (*Figure 1*), permitting oxytocin and prostaglandin promotion of contractions. There is extensive evidence suggesting that hCG may function prior to implantation of pregnancy. hCG has been found in sperm, and hCG/LH receptors found in fallopian tubes (68, 71, 76). Interestingly, it has been demonstrated that blastocysts use hCG to signal the decidua that they are awaiting implantation, prior to implantation (80, 87, 89). This strange interuterine space signaling may prepare the decidua for implantation. What promotes fetal growth during pregnancy has always been a conundrum. Growth hormone is inactive in pregnancy, and hPL and hPGH are maternal hormones and have very limited action on fetal growth. Multiple recent studies by different investigators show that fetal organs contain hCG receptors (61, 72, 79, 83, 89). It is inferred that hCG is the fetal growth factor and that

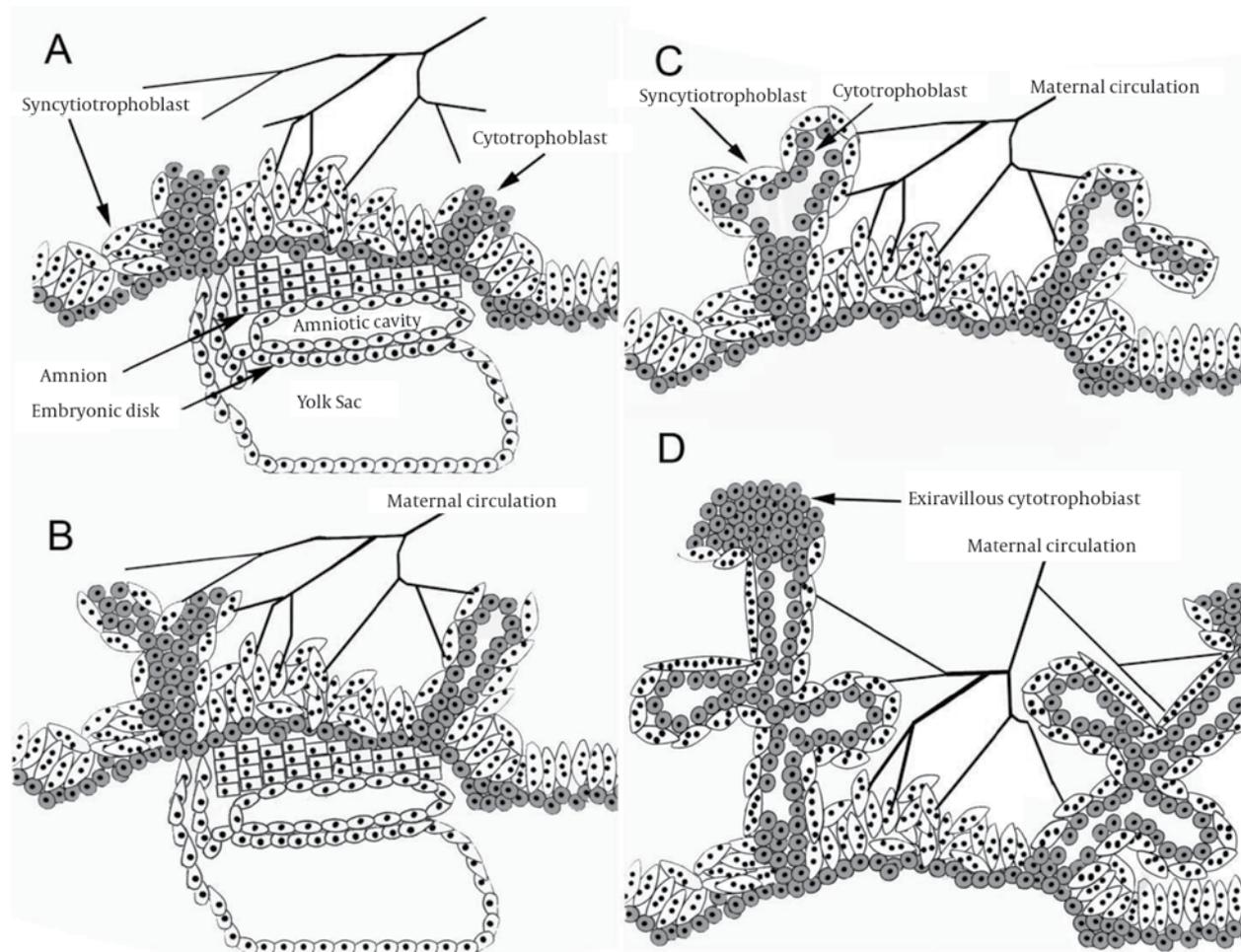


Figure 4. hCG Function in Villous Trophoblast Tissue Formation.

Hyperglycosylated hCG function to promote cytotrophoblast tissue synthesis (15, 16, 37-40), hcg then function to promote fusion and differentiation of this tissue to multinucleated syncytiotrophoblast cells (90).

Panel A shows cytotrophoblast columns in conceptus placental tissue promoted by hyperglycosylated hCG. As shown in Panels B and C columns of cytotrophoblast cell protrude from the tissue, this differentiates around the edges, promoted by hCG, to form syncytiotrophoblast tissue. As a result of this process villous structures are formed (Panels C and D).

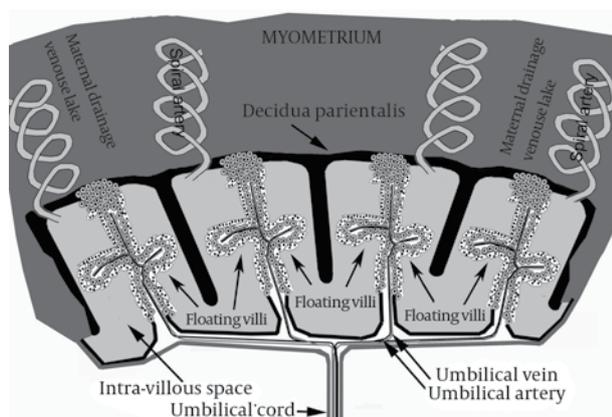


Figure 5. Ultra-Efficient Fetal-Maternal Nutrient Exchange or Hemochorial Placentation. hCG and hyperglycosylated hCG promoted villous placenta formation as indicated in figure 4. hCG promotes growth or angiogenesis of uterine arteries to meet the growing placenta (24, 64, 74, 92, 93, 97, 98). hCG also controls the growth of umbilical vasculature to meet the developing hemochorial placentation. All told, hCG and hyperglycosylated hCG function to build ultra-efficient nutrient exchange or hemochorial placentation.

hCG controls fetal growth during pregnancy. Fetal hCG receptor disappear prior to parturition so are not present in human infants. The fetus has been demonstrated to produce its own variant of hCG in the fetal liver and kidney (61). Control of fetal growth may be one of these most major functions of hCG.

The story does not end here, hCG receptor have been identified in maternal brain hippocampus, brain stem and brain hypothalamus. We do not understand brain function well enough to understand the function of hCG in human brain. Maybe there are some maternal brain adjustments associated with pregnancy? We do realize a likely symptom of the brain receptors in key locations in the brain, that is hyperemesis gravidarum or the cause of morning sickness or nausea and vomiting during pregnancy (79, 83, 85). Let us consider hyperglycosylated hCG, that cytotrophoblast autocrine produced during pregnancy. Hyperglycosylated hCG is a form of hCG with large triantennary N-linked oligosaccharides (Figure 3)

and hexasaccharide O-linked oligosaccharides (42, 43). It is our understanding that hyperglycosylated hCG exposes hCG's common structure with TGF β (23) and antagonizes a TGF β receptor on cytotrophoblast cells (15). The binding to TGF β receptor blocks apoptosis in cells (73), promotes cell growth and cell production of invasive enzymes, metalloproteinases and collagenases (99-104). Hyperglycosylated hCG is the principal form of hCG produced in early pregnancy because it drives implantation of pregnancy (37, 40), using the metalloproteinases and collagenases to digest the way of blastocyst/conceptus/placenta as it invades deep into the uterus. By the end of the first trimester of pregnancy the placenta buries itself at approximately 1/3rd the thickness of the myometrium [108], having penetrated the decidual and the myometrium. Hyperglycosylated hCG drive placenta or cytotrophoblast cell growth during the course of pregnancy (15, 16, 24, 37, 64, 74, 90, 92, 93, 97, 98), cytotrophoblast cells are continuously fused and differentiated to make syncytiotrophoblast cells under the control of hCG (90). Hyperglycosylated hCG may drive implantation of pregnancy (37, 40), yet unfortunately invasion-like processes are not always perfect. Ultra-efficient hemochorial placentation developed in humans to permit the evolution of the human brain (18, 65). Such demand for ultra-efficiency comes with valves and failures. As shown, inefficient pregnancy implantation is the cause of all spontaneous abortions or miscarriages of pregnancy, except for gross genetic abnormalities (105, 106). It is also the cause of biochemical pregnancies (105, 106). As now demonstrated, these are all due to deficient hyperglycosylated hCG (107). As shown, when hyperglycosylated hCG is less than 50% of total hCG at the time of implantation, a biochemical pregnancy or spontaneous abortion is the outcome (107) This study was quite absolute, 81 of 81 pregnancy with term outcome has a hyperglycosylated hCG of >50% of total hCG (107). Hyperglycosylated hCG (<50%) is seemingly a definitive test for pregnancy outcome on the day of pregnancy implantation. Hyperglycosylated hCG is also valuable during pregnancy in the detection of Down syndrome pregnancies. Hyperglycosylated hCG, the implantation driver, outperforms hCG and its free β -subunit in detecting Down syndrome pregnancies (108,

109). Hyperglycosylated hCG is also useful in the prediction of preeclampsia or pregnancy induced hypertension in pregnancy (110). Preeclampsia or pregnancy induced hypertension result when hemochorial placentation fails to be instrumented correctly due to failure of complete placental invasion/implantation (111, 112).

3. hCG and Hyperglycosylated hCG, the Potent Growth and Invasion Factors That Forced Human Evolution

It is fascinating to examine how and why hCG and hyperglycosylated hCG evolved. They appeared to evolve as ultra-growth and invasion factors, that seemingly promoted placentation in primates to the extreme level needed to support the development of the human brain (113-115). It is these ultra-growth and invasion factors that drive human pregnancy or human life, and are abused by human cancers as described in this report, to drive them through deadly malignancy. That is why in this review's title we call hCG the centerpiece of life and death.

This section summarizes publications describing the role of hCG and hyperglycosylated hCG in human evolution (18, 65, 116). The earliest primates, prosimian primates (i.e. lemurs) had small brains like earlier mammals, 0.07% of body weight (Table 4). They had small brains because these primates and earlier mammals used an ineffective placentation system, non-invasive epitheliochorial placentation. In this placentation system, maternal nutrients travelled across multiple layers of decidua, endometrial epithelium, chorionic epithelium and connective tissue to reach the fetal circulation or the umbilical veins. With evolution, the next level of primates, early simian primates (i.e. marmoset and monkey) CG (we call it CG rather than hCG, since this is not humans) and hyperglycosylated CG first evolved. A long with the evolution of these molecules came their functional product, hemochorial placentation, as described in section 2 of this review on human pregnancy (15, 16, 37-39, 65, 68, 73, 87, 88, 94). Hemochorial placentation, or the maternal-fetal circulation interface, was much more efficient than epitheliochorial placentation, with nutrients only having to cross one cell thickness, syncytiotrophoblast cells, to cross between the maternal circulation and umbilical of

Table 4. Parallels Between Placental Implantation and Invasion Characteristics in Primates, Presence and Sugar Structure on Chorionic Gonadotropin (CG) or LH, and Relative Brain Masses.

| | Placentation | Depth of Invasion of Placenta | Sugar Structures, Acidity or pI ^a | Brain Mass (% Body Weight) | First Appearance |
|--------------------------|------------------|-------------------------------|---|----------------------------|-----------------------|
| Humans | Hemochorial | 1/3rd myometrium | CG, 8 oligosaccharides, pI 3.5 | 2.4 | 0.1 million years ago |
| Advanced simian primates | Hemochorial | 1/10th myometrium | CG, 6 oligosaccharides, pI 4.9 | 0.74 | 20 million years ago |
| Early simian primates | Hemochorial | through decidua only | CG, 5 oligosaccharides, pI 6.3 | 0.17 | 37 million years ago |
| Prosimian primate | Epitheliochorial | no-invasion | No CG produced, only LH LH, 3 oligosaccharides, pI 9.0 | 0.07 | 55 million years ago |

^a Isoelectric Point

fetal circulation. The story of CG and hyperglycosylated CG and evolution is how an ultra-efficient form of hCG evolved, capable of forming ultra-efficient hemochorial placentation that permitted the evolution of the human brain.

In 1980 Fiddes and Goodman (19) examined the DNA sequence for the β -subunits of CG and LH in humans and primates. They showed that the evolution of CG from LH occurred in early simian primates by a single point deletion mutation in LH β -subunit DNA at a base corresponding to LH amino acid residue 114, and read-through into the 3'-untranslated region of the molecule making and β -subunit of 145 vs. 121 amino acids. In 2002 Maston and Ruvolo (117) investigated the DNA sequences of the β -subunits of CG in 14 different primates and showed that the genes to make CG and its variants were not present in prosimian primates. They confirmed that CG evolved by the indicated deletion mutation with early simian primates.

While early simian primate LH had just 3 N-linked oligosaccharides, the first or early simian primate CG and hyperglycosylated CG molecules had 3 N-linked and 2 O-linked oligosaccharides or 5 oligosaccharides total, with the 2 O-linked oligosaccharides developing from the extended peptide sequence, the β -subunit C-terminal peptide, deriving from the read through into the 3'-untranslated region of the LH β gene (Table 4).

Early simian primates evolved approximately 37 million year ago (Table 4). With the evolution of advanced simian primates, about 20 million ago (examples: orangutan and chimpanzee) came with further point mutations a form of CG and hyperglycosylated CG that had 3 N-linked and 3 O-linked oligosaccharides or 6 oligosaccharides total (Table 4). With the evolution of humans, 0.1 million year ago, and with multiple further point mutations came the evolution of human CG (hCG) and hyperglycosylated CG (hyperglycosylated hCG) having 4 N-linked and 4 O-linked oligosaccharides or 8 oligosaccharides total. With the increasing numbers of oligosaccharides and their terminating sialic residues (2 per oligosaccharide) or acidic sugars, and with structures evolving with 3 oligosaccharides (6 sialic acid), 5 oligosaccharides total (10 sialic acid), to 6 oligosaccharides total (12 sialic acid) and on to 8 oligosaccharides total (16 sialic acid) came the evolution of a human CG with a greater and greater acidity. The acidity ranged from isoelectric point (pI) 9.0 with prosimian LH to pI 6.3 in early simian CG, to pI 4.8 in advanced simian CG and on to super-acidic pI 3.5 molecules in human CG (18, 65, 117-119). CG is the most acidic glycoprotein made by humans. Evolution took hCG acidity to the extreme.

The metabolic clearance rate of circulating levels of CG advanced with evolution and acidity. As CG evolved with further oligosaccharides containing sialic acid, it very much lengthened the metabolic clearance rate or biological activity of CG and hyperglycosylated CG molecules (117, 120, 121). As an example, at one extreme is LH [pI 9.0

(49)] with just 3 N-linked oligosaccharides. This has a pI of 9.0 and a circulating half-life of 25 minutes (49). At the other extreme is regular human CG with 8 oligosaccharides terminating in sialic acid. These acidify hCG resulting in a molecule with a mean pI of 3.5, and a circulating half-life of 2160 minute (34). Effectively the extreme acidity of hCG extends its biological activity 2160/25 or 86-fold over LH.

Considering that LH has 3 oligosaccharides and a clearance rate, half-life of 25 minutes, and human CG has 8 oligosaccharides and a clearance rate half-life of 2160 minutes, a regression equation was found linking the number of oligosaccharides and the metabolic clearance rates of molecules. If clearance rate is CR and number of oligosaccharides is #O then $CR = 2.4\#O \times 1.9$. It was calculated using this equation that while the clearance rate half-life of LH and human CG are 25 and 2160 minutes, that the clearance rate half-life of early simian primate CG was roughly 150 minutes and the clearance rate half-life of advanced simian primate CG was 360 minutes. As such, human CG is approximate 6-fold more potent than advanced primate CG, which is approximately 2.4-fold more potent than early simian CG, which was approximately 6-fold more potent than LH.

The size of the brain in mammals is related to the combination of body mass and the metabolic support of the developing offspring (112-118, 122-126). The larger brain seen in advanced primates and humans, correlates with disproportionately large energy demands by the developing fetuses [128-130]. Many studies support the concept that advanced primates and humans had to develop ultra-efficient placentation mechanisms to support the increasing nutritional demands of their embryonic brain (Table 4) (118, 119, 122-127).

Prosimian primates had an average mammalian brain, 0.07% of body mass (Table 4). In this species, epitheliochorial placentation was sufficient. Hemochorial placentation started with the evolution of CG in early simian primates. It was only with the appearance of CG and hyperglycosylated CG in early simian primates, that the signals to implant placentas inside the uterus (37-40), and to generate villous placenta (18, 65, 116), to promote angiogenesis of uterine vasculature (24, 64, 74, 92, 93, 97, 98) and development of the umbilical cord (70, 96) that hemochorial placentation happened (65, 116). Hemochorial placentation was primitive in early simian primates, placentas implanting only through the depth of the decidua, leading to a larger brain 0.17% of body mass (Table 4). It was with evolution and the development of more-acidic more-potent CG and hyperglycosylated CG in advanced simian primates that hemochorial placentation went deeper to 1/10th of myometrial depth in advanced simian primates (Table 4). Hemochorial placentation supported the development of a much larger brain in advanced simian primates of 0.74% of body mass (Table 4).

With the evolution of humans and the multiple mutations needed to produce their super acidic-CG with

2 additional oligosaccharides, one N-linked and one O-linked, hemochorial placentation took the molecule to the extreme. CG jumped in acidity from pI 4.8 to pI 3.5, this raised the metabolic clearance rate half-life from 360 minutes (6 hours) to 2160 minutes (36 hours). With this hemochorial placentation went deeper to 1/3rd the width of the myometrium (Table 4). Hemochorial placentation reached then the ultra-efficiency needed to support a human brain, 2.4% of body mass.

Nutrition transfer and placentation was taken to the extreme in the humans. Human CG is extremely acidic with 4 O-linked and 4 N-linked oligosaccharides with a circulating half-time of 2160 minutes. This causes invasion through the decidua to one third the thickness of the myometrium and to the ultra-efficient placentation that is needed to support the nutritional transfer necessary for a brain of 2.4% body mass or 3 fold greater than that of advanced simians, to develop (113-115, 127). Considering the relationship between regular CG, hyperglycosylated CG and hemochorial placentation, and between advancing acidity of CG and advancing invasion and angiogenesis, it would be appropriate to claim that the evolution of CG in early simians started primates on the evolution path to advanced brains, or is at the root of the evolution of humans (18, 65, 116).

It is with this evolution story that human ultra efficient-CG and ultra efficient -hyperglycosylated CG were created. Unfortunately, human cancers abuse these ultra-efficient growth, angiogenic and invasion factors to drive malignancy (see Section 5, hCG and cancer). This should be considered an artifact of human evolution.

4. Sulfated hCG a Key Hormone Produced by the Pituitary Gland

A fifth variant of hCG is produced by pituitary gonadotrope cells during the routine menstrual cycle in women, this is sulfated hCG (31, 128). It is wrong to say that hCG is strictly a pregnancy hormone, it is normally produced in pregnancy and in normal women during the menstrual cycle. Non-pregnancy production of hCG has been a major source of confusion in the medical field. Research using a sensitive total or intact hCG assay shows that sulfated hCG is secreted during the length of the menstrual cycle (31), analogous to the normal secretion pattern of LH. Sulfated hCG and LH both bind a joint receptor, the hCG/LH receptor. Pituitary hCG contains a mixture of sulfated and sialylated oligosaccharides (Figure 2) (9). Research shows that in 277 menstrual cycles at the time of the mid-cycle LH peak sulfated hCG production averages 1.54 ± 0.90 mIU/mL (129). Sulfated hCG copies LH actions in promoting progesterone production by corpus luteal cells, androstenedione production by theca cells, and in enhancing ovulation. LH, FSH and sulfated hCG, produced by gonadotrope cells, all have key functions during the menstrual cycle (15, 31, 128, 129).

Why we ask does the pituitary produce sulfated hCG to

supplement LH and to help LH complete the LH job? Sulfated α -subunit is produced in excess by the pituitary. A single α -subunit pairs with hCG, LH, follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH). Gonadotropin releasing hormone (GnRH) is made by the hypothalamus of the brain and through the hypothalamic-hypophyseal portal circulation promotes the β -subunits of LH and FSH. As illustrated in Figure 6, a single LHB gene resides directly next to 8 tandem genes coding for hCG β on chromosome 19. Since such a tiny amount of hCG are produced by the pituitary it is thought that hCG β is expressed as consequence of large pulses of GnRH acting on the LHB gene. Clearly, the large pulses of GnRH cannot avoid promoting hCG β (Figure 6).

It has been calculated that sulfated hCG (0.01 – 9 mIU/ml) is produced at approximately 1/50th the serum concentration of LH (1 – 159 mIU/ml) (9, 31, 129). Yet sulfated

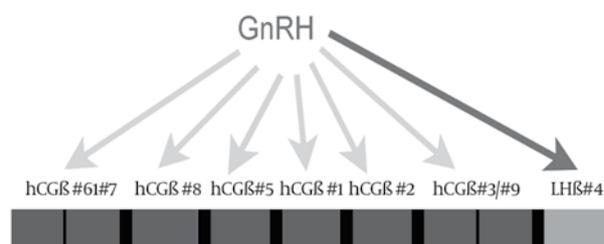


Figure 6. Mechanism of Action of GnRH in Production of Sulfated Pituitary hCG. Pulses of GnRH Promote LHB Gene, but cannot avoid stimulating associated hCG β genes.

hCG has 50 times the biological activity of LH per mole of hormone released in blood (sulfated hCG is less potent than regular hCG, which is 86-fold more potent than LH) (9). Considering the 2 calculations together, 1/50th the concentration and 50-fold greater biological activity, sulfated hCG and LH seemingly have equal functions. Equal functions in promoting androstenedione production, promoting progesterone production and promoting ovulation. No longer can the claim be made that LH promotes ovulation, it is LH and sulfated hCG that jointly promote ovulation. Some cases have low LH at ovulation. Without measuring sulfated hCG and LH together a true assessment of ovulation problems cannot be obtained. Sulfated hCG at 1/50th the concentration of LH is barely detectable during the menstrual cycle. At the time of the LH peak, however, hCG level averaged 1.54 ± 0.90 mIU/mL (129), or was mostly detectable by modern total hCG immunoassays. In menopause, with the absence of sex steroid feedback (estradiol and progesterone) to the hypothalamus, GnRH pulses from the hypothalamus become maximal. The result is promotion of excess LH, hCG and FSH production by gonadotrope cell due to extreme GnRH pulses. Serum LH increases from, 1-90 mIU/mL to >100 mIU/mL in menopause, serum FSH increases from 1-29 mIU/ml to 30-200 mIU/ml in menopause, and serum hCG from <0.01 – 9 mIU/ml to 2-39 mIU/ml in menopause. All told, normal sulfated hCG is very detectable in a menopausal woman (9, 31, 128-131). Perimenopause is the

stage prior to menopause marked by oligomenorrhea or irregular menstrual periods. Just as perimenopause is marked by the start of raised LH and FSH production, it is also marked by abnormal detectable hCG levels. Seemingly, exceptional women can achieve pregnancy up to 60 years age. This is very rare. In general, women 40-55 years age are likely to be in perimenopause, and those >55 years age are surly in menopause (132). As reported, the range of hCG detection in non-pregnant menopausal women, >55 years age, is <2 to 13.1 mIU/ml, in non-pregnant menstrual women 18-40 years age is <2 to 4.6 mIU/ml, and potential perimenopause women is <2 to 7.7 mIU/ml (132). In the USA hCG Reference Service experience, hCG levels as high as 29 mIU/ml, median 7.2 mIU/ml are detected in perimenopause and as high as 33 mIU/ml, median 8.0 mIU/ml are detected in menopause. Higher hCG levels have been recorded, as high as 39 mIU/ml, in women having oophorectomy or thrown into amenorrhea and menopause.

Most medical practices and hospitals throughout the world check women in an hCG test prior to the use of certain drugs, certain invasive procedures, and always prior to surgery. No care is given regarding women in perimenopause or menopause who may be naturally producing sulfated pituitary hCG. Unfortunately, when these women show positive for hCG it raises a needless alarm amongst doctors, leading to cancellation of surgery or other procedures. Physicians check these women first for the fetal sac of pregnancy by ultrasound, then after excluding clinical pregnancy they consider ectopic pregnancy. I have published dozens of times in journal ranging from New England Journal of Medicine to Clinical Chemistry (130, 131) that a positive hCG test in a woman over 45 is normal and should not be a concern. It is easy to prove that hCG production in a woman over 45 is pituitary hCG and very normal. Place a woman on a high estrogen contraceptive pill (Ortho-Novum, 0.035 mg ethinyl estradiol) for 3 weeks, if her hCG then becomes undetectable, it is pituitary hCG.

Gronowowski et al. (133), showed that measurement of FSH levels is a powerful indicator of pituitary sulfated hCG (FSH >30 mIU/ml). The USA hCG Reference Service started using FSH as a confirmation of pituitary sulfated hCG about one year ago. As found, all menopausal cases tested since we started measuring FSH, are highly positive for FSH (61-190 mIU/ml). The high 190 mIU/ml FSH correlated with the case producing the highest pituitary sulfated hCG, 39 mIU/ml. We very much support the use of FSH testing to confirm pituitary hCG. Once a woman is diagnosed as producing pituitary sulfated hCG, what do you do next? The answer is "nothing," it is normal it is natural, you need to completely ignore it.

5. Hyperglycosylated hCG, hCG Free β , and Hyperglycosylated Free β , the Potent Growth and Invasion Factors Abused by Human Malignancies

The preceding sections describe the key roles hyperglycosylated hCG has in commanding pregnancy. As describe in Section 3, this extreme growth and invasion factor was generated to drive human evolution. While it is very acceptable that this extreme factor goes on to drive human pregnancy, it is not acceptable that this factor is abused to drive all advanced human malignancies. Hyperglycosylated hCG and its analogues are seemingly selected as potent TGF β antagonist (15). Evolutionarily selected to use this potent invasive pathway to drive human malignancies (13, 18, 65). Here we carefully examine what we now know about these molecules and how they seemingly drive all human cancers. Multiple investigators have noticed a common molecular evolutionary origin of TGF β and hCG β (134, 135). In 1994 Laphorn and colleagues (23) found a rare four peptide linked cystine knot structure within the 3 dimensional structure of hCG β . This cystine knot structure is common to hCG β and TGF β . As found by Butler and colleagues in 1990 (13), hCG free β , a proven cancer promoter (13, 15, 17, 136-160), acts on cancer by antagonizing the TGF β receptor on cancer cells. As shown recently, hCG does not express these TGF β binding structures and does not function by this pathway (15), but hyperglycosylated hCG (choriocarcinoma cancer promoter (16, 37)), and another cancer promoter, hyperglycosylated free β , both are interchangeable with hCG free β in promoting growth of cancer cells. Indicating that all 3 interchangeable promoters, hCG free β , hyperglycosylated hCG and hyperglycosylated free β function by antagonizing a TGF β receptor (15). The antagonism of a TGF β receptor blocks apoptosis in cancer cells (13, 73, 139, 140, 148), promotes cancer cell growth and cell production of invasive enzymes, metalloproteinases and collagenases (13, 99-104, 137, 139, 140, 145, 148, 150, 153). History identifies two hCG-related cancer markers, hCG free β and hyperglycosylated hCG free β . Many researchers noted through the years that the hCG free β produced by cancer cell lines and present in cancer patient serum was unduly large (17, 158), larger than hCG β -subunit in size. In 2006 Valmu and colleagues (43) used mass spectrometry to show that the large free β is actually hyperglycosylated free β , resembling the β -subunit of hyperglycosylated hCG. Recently, it has been shown that cancers invariably produce different proportions of hCG free β and hyperglycosylated free β (15). It is thought that cancer cell free β is invariably glycosylated containing 0-100% hyperglycosylated free β . Is it necessary to keep calling these free β and hyperglycosylated free β . I think not, maybe they should be called vg (variably glycosylated) hCG free β (vg-hCG free β). Choriocarcinoma and germ cell malignancies characteristically produce cytotrophoblast cells and express hyperglycosylated hCG (15, 37). It is my understanding that hyperglycosylated hCG drives all these malignancies, by the same TGF β antagonizing pathways that hyperglycosylated hCG used to implant pregnancies. Hyperglycosylated hCG drives all stages of these malignancies, early and advanced (161, 162). As demonstrate, when these malignancies are

transplanted into nude mice they grow and metastasize rapidly. When the nude mice are given injections of B152 monoclonal antibody to hyperglycosylated hCG all growth and malignancy comes to a complete halt. Effectively, blockage of hyperglycosylated hCG cures or blunts these diseases (16, 73). We call these cancers Type 1 cancers, or those totally dependent on hyperglycosylated hCG. Starting in the early 1970s with the introduction of the hCG β radioimmunoassay (33) scientist started showing that all cancer cell lines produced an hCG β immunoreactive molecule, and hCG β immunoreactive molecules were detected in cancer patient serum and urine. In 1978 Franchimont and colleagues (21), and in 1980 Rosen and colleagues demonstrated an hCG free β produced by a cancer cell line. In 1983 I demonstrated hCG free β -subunit in two cervical cancer cell lines and then found an hCG free β in corresponding cancer cases serum and urine (17). As found at that time, the hCG free β was unduly large, recently shown to be hyperglycosylated free β . We now call all of these cancer molecules vg-hCG free β .

Reports by the hundreds continued showing that cancer cell lines produced vg-hCG free β or an hCG β -immunoreactive molecule (136-160). Between 1992 and 1996 Acevedo and colleagues (4, 136, 163) and Regelson and colleagues (156) showed using flow cytometry and immunoassay methods that all cancers made vg-hCG free β . Interestingly, in a large compilation of studies, shown in Table 5, vg-hCG free β and its urine degradation product β -core fragment can only be detected in 30% (serum vg-hCG free β) and 48% (urine β -core fragment) of cancer cases. How can we explain this discrepancy, 30% and 48% versus 100%? The problem is that vg-hCG free β is normally very rapidly cleared from the circulation (34). When one considers that monocytes, leukocytes and macrophages accumulate close to a malignancy, cleaving the vg-hCG so that it is nicked and has a cleaved C-terminal peptide, then clearance from the circulation may be reduced to seconds (45). As a result, these tumor vg-hCG free β molecules and their degradation product β -core fragment, may only rarely be detectable in serum or urine. Considering the detection of 30% and 48% of cases, seemingly

the extreme cases, these findings are consistent with all cancers producing vg-hCG free β .

In recent years it has been demonstrated that vg-hCG free β has key roles in advanced malignancies, promotes cancer growth and malignancy (13, 137, 139, 140, 145, 148, 150, 153). Acting by antagonizing the cancer cell TGF β receptor (13), vg-hCG free β seemingly blocks cell apoptosis (13, 139, 140, 148). Much more research has been completed on how the interchangeable marker hyperglycosylated hCG, antagonizes a TGF β receptor and promotes implantation of pregnancy (15) using metalloproteinases and collagenases (99-104). It is inferred that TGF β antagonism in cancer cell similarly enhanced metalloproteinases and collagenase production, leading to cell invasion or metastases. Today we know that vg-hCG free β enhancement of all cancers is limited to advanced malignancies only. It appears that cancers advance using alternative growth and invasion mechanisms. When cancer advance mechanism are found to express the hCG β genes, 8 tandem genes, on chromosome 19. With the expression of vg-hCG free β a new efficient pathway is found by the cancer cell to promote cancer growth and invasion, the vg-hCG : TGF β antagonism pathway. It is clear from the vaccine studies, described below, that this becomes the dominant or solitary cancer growth and invasion pathways.

How are all these cancer discoveries reflecting on cancer treatment? Three companies, Celldex, CG Therapeutics and MCI BioPharma Inc. started in 2000 testing a synthetic hCG β vaccine in treating advanced cancer cases (164-169). Results are very exciting, with the finding that hCG β vaccines are considerably extending lives of advanced cancer patients. For example, examining the clinical trial with hCG β vaccine and colorectal cancers (168), the average survival of those with optimal antibody response was 45 weeks, compared to just 24 weeks in those without optimal antibody response ($p = 0.0003$). This demonstrates that hCG antibodies can double longevity. Similar results have been reported with prostate cancer, lung cancer and breast cancer. We ask, could the discovery of vg-hCG free β be the new cancer cure?

Table 5. Use of Serum Free β -Subunit (vg-hCG Free β) and Urine β -Core Fragment as Tumor Markers for Detection of Malignancies. All averages are determined by combining total positive cases from multiple reports (136-160).

| | hCG β as a Tumor Marker | | β -Core Fragment as a Tumor Marker | |
|--------------------|-------------------------------|----------------------------------|--|---|
| | Cases, No. | Serum hCG β (>3 pmol/L), % | Cases, No. | Urine β -Core Fragment (>3 pmol/L), % |
| Bladder cancer | 170 | 35 | 102 | 48 |
| Cervical cancer | 60 | 37 | 410 | 48 |
| Colorectal cancer | 436 | 17 | | |
| Endometrial cancer | 55 | 33 | 157 | 47 |
| Lung cancer | 243 | 18 | 122 | 45 |
| Ovarian cancer | 150 | 38 | 207 | 66 |
| Pancreatic cancer | - | - | 29 | 55 |
| Vulvar | 64 | 41 | - | - |
| TOTAL | 1164 | Mean 30 | 1027 | Mean 48 |

The company Omnimune Corp. is proposing to generate human antibodies to hyperglycosylated hCG/hCG β and to hCG β . These antibodies should generate a higher success rate than the vaccine since they do not require the limitation of having appropriate immunity, which a person undergoing multiple cancer chemotherapies may not have. If you consider that a vaccine can raise human survival 2-fold in advanced malignancies then what may be on the horizon using administered antibodies, maybe more than 2-fold improvement of survival for advanced cancer cases. Raising human advanced cancer survival more than 2-fold brings cancer treatment into a new horizon.

I consider general malignancies as type 2 malignancies in that vg-hCG free β is only critical to advanced malignancies. I believe that the hCG and cancer story will bring a promising future to cancer therapy. Please remember, this just an artifact to human evolution. I hope that in the near future that hCG β antibody cancer therapy may bring a cure to type 1 malignancies, and a great improvement in therapy for type 2 malignancies.

Acknowledgments

None declared.

Financial Disclosure

None declared.

Funding/Support

None declared.

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