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Unravelling the Connection Between Gestational Diabetes Mellitus and Butyrylcholinesterase

Alexander E. Omu Frcog
Department of Obstetrics and Gynaecology, Faculty of Medicine, Kuwait University, Kuwait

1. Introduction

Gestational Diabetes Mellitus (GDM) is glucose intolerance first diagnosed during pregnancy (Metzger and Coustan 1998, World Health Organization 1999). Gestational diabetes is a condition that complicates 3-12.% of pregnancies (Gabbe and Graves 2003, Omu et al 2010) with wide variation in the incidence of gestational diabetes reported among ethnic groups.This could be newly diagnosed type 1 or type 2 Diabetes Mellitus or a new onset of hyperglycemia secondary to metabolic changes related to pregnancy (Yogev and Visser 2009). The rates of Gestational diabetes mellitus are increasing with the epidemic of obesity worldwide. Risk factors for GDM include advanced maternal age, multiparity, and racial or ethnic minority status (Table 1).

• Body mass index more than 30 kg/m²
• Previous macrosomic baby weighing 4.5 kg or more
• Previous gestational diabetes
• Family history of diabetes (first-degree relative with diabetes)
• Family origin with a high prevalence of diabetes:
• South Asian (specifically women whose country of family origin is India, Pakistan or
• Bangladesh)
• Black Caribbean
• Middle Eastern (specifically women whose country of family origin is Saudi Arabia, United Arab Emirates, Iraq, Jordan, Syria, Oman, Qatar, Kuwait, Lebanon or Egypt)

Reproduced from the National Institute for Health and Clinical Excellence guideline for diabetes in pregnancy (12) by RCOG Scientific Advisory Committee Opinion Paper 23: Diagnosis and Treatment of Gestational Diabetes (RCOG 2011).

Table 1. Risk factors for Gestational diabetes mellitus

There are health implications for both the mother and infant who remain at risk for complications such as embryopathies, spontaneous abortion and perinatal mortality and morbidity (Loeken 2006).
There has been considerable controversy surrounding the screening and diagnosis, natural history, management and outcome of women with gestational diabetes. The 2008 NICE guidelines (NICE 2008) on diabetes in pregnancy detailed a screening programme targeting biochemical screening to women with risk factors. Women with a history of gestational diabetes mellitus (GDM) have an increased risk for recurrence in subsequent pregnancies, according to the results of a population-based, retrospective cohort study (HAPO Study Cooperative Research Group 2008). Oxidative stress has been implicated in the pathogenesis and development of complications of diabetes in pregnancy (King and Loeken 2004, Loeken 2004, Marfella et al 2001, Morgan et al 2008, Rosen et al 2001, Wender-Ozegowska et al 2004). The role of Butyrylcholinesterase (BuChE) in the aetiology, screening and monitoring, complications and future drug development of Gestational Diabetes Mellitus has become an interesting area of speculative research (Mahmoud et al 2003, Mohmoud etal 2006, Mahmoud et al 2008, Rustemeijer et al 2001, Serlin et al 2009, Sternfield et al 1997).

2. The objective

The objective of this chapter is to elucidate the relationship between gestational diabetes mellitus (GDM) and BuChE in the pathogenesis, monitoring and future drug development.

3. Pathogenesis of GDM

The cornerstones of development of gestational diabetes mellitus are related to modern lifestyle, principally, a lack of exercise and an unhealthy diet, the environment and some degree of genetic profile (American Diabetic Association 2003, Hollander et al 2007, Serlin et al 2009). Elevated glucose in pregnancy may be caused by increased levels of diabetogenic factors of pregnancy such as glucocorticoids, human placental lactogen and oestrogens. Hyperglycaemia causes oxidative stress due to increased production of mitochondrial ROS, nonenzymatic glycation of proteins, and glucose autoxidation (Brownlee 2001). Elevated FFA can also cause oxidative stress due to increased mitochondrial uncoupling and ß-oxidation, leading to the increased production of ROS. In addition, hyperglycemia- and FFA-induced oxidative stress leads to the activation of stress-sensitive signaling pathways (Evans et al 2003). This, in turn, worsens both insulin secretion and action, leading to overt Gestational Diabetes Mellitus. Administration of glucocorticoids significantly decreases the catalytic activity of BuChE in plasma and liver regardless of sex (Vrdoljaki et al 2005).

3.1 Hyperglycemia and oxidative stress

The pathogenic effect of hyperglycaemia is mediated to a significant extent via increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and subsequent oxidative stress (King and Loeken 2004, Marfella2001, Rosen et al 2001, Wender-Ozegowska et al 2004). ROS and RNS directly oxidize and damage DNA, proteins, and lipids and thus adversely affect the pancreas especially the β Langerhan cells that produce insulin. Similarly, there is disruption of the alpha cells that produce glucagon. The paracrine relationship between the pancreatic beta and alpha cells is disrupted to cause β-cell dysfunction. There is also growing evidence that activation of stress-sensitive pathways, such

as NF-kB, p38 MAPK, JNK/SAPK, and hexosamine, by elevations in glucose and possibly FFA levels leads to both insulin resistance and impaired insulin secretion through ß-cell dysfunction (Kyriakis et al 1992). Circulating serum levels of lipid peroxidation product malonedialdehyde (MDA) and protein oxidation markers are elevated in GDM compared to healthy normal pregnancy and give rise to a negatively strong correlation between MDA and BuChE in serum and placenta (Omu et al 2010). A third causal pathway may be through the induction of apoptosis of the beta cells by advanced glycation end-products. This would explain the varying severity of GDM among the patients. BuChE deficiency results in delayed metabolism of a number of compounds of clinical significance, including glucose, thus contributing to the pathogenesis of diabetes mellitus. Glucose metabolism is controlled by the hormone insulin produced in the pancreas. BuChE deficiency in pregnancy may be as a result of hereditary deficiency and haemodilution in second half of pregnancy.

3.2 Role of estrogen receptor alpha in glucose and lipid metabolism

The estrogen receptor ER-alpha is emerging as a key molecule involved in glucose and lipid metabolism. The activation of ER-alpha by physiological concentrations of E2 may play an important role in the adaptation of the endocrine pancreas to pregnancy. However, if ER-alpha is over stimulated by an excess of E2 or the action of an environmental estrogen such as Biphenol A (Nadal et al 2009, Paloma et al 2008, Ropero et al 2008), it can result in an excessive insulin signaling. This may provoke insulin resistance in the liver and muscle, as well as beta-cell exhaustion and therefore, contribute to the development of Gestational Diabetes. An association between oestrogen receptor alpha and BuChE has been reported (Combarros et al 2007).

3.3 Environmental factors

The increase of endocrine-disrupting chemicals (EDCs) in the environment has been implicated in the aetiology of GDM (Elobeid and Allison 2008, Newbold et al 2009, Rubin and Sato 2009). A connection at the epidemiologic level in humans has been recently proposed for dioxin, an environmental contaminant that acts through other than estrogen receptors (ERs) as an endocrine disruptor (Bertazzi et al 2001, Remillard et al 2002).

3.4 Autoimmunity and Treg in GDM

Autoimmune phenomena associated with type 1 diabetes mellitus (DM) can also be detected in a subgroup of women with GDM. Islet autoantibodies are present in sera from women with GDM with variable frequency. Distinct phenotypic and genotypic features may be recognised in this subset of women with GDM, which are representative of a distinct clinical entity. Women with previous autoimmune GDM may be candidates for potential immune intervention strategies (Mauricio et al 2001). Normal activity of Treg subpopulations are disrupted in GDM by mechanisms that threaten pregnancy and may contribute to other features of the disorder, with higher percentages of activated T cells than a matched population of healthy pregnant women. BuChE detoxifies anticholinesterases (AC) that are known to threaten pregnancy and one or more of these fetotoxins adversely impacts pregnancy outcome through a mechanism that may include Treg cells. In normal pregnancy, there is correlation between Treg activity and BuChE, whereas in women in whom adequate correlation between Treg cells and BuChE activity is not achieved (Mahmoud et al 2003, Mahmoud et al 2006, Mahmoud et al 2008, Saito et al 2005), there is

failure of effective clearance of toxicants which adversely affect maternal immunomodulation in ways that can lead to GDM and other pregnancy-threatening conditions (Baccarelli et al 2002, Bertazzi et al 2001, Eskenazi et al 2004, Lappas et al 2010, Maussolie et al 1992, Remillard et al 2002).

3.5 Genetics of gestational diabetes mellitus

There is very little published data about the genetic basis for gestational diabetes mellitus (GDM) (Watanabe et al 2007). However, there is evidence for clustering of type 2 diabetes and impaired glucose tolerance in families with a GDM (McLellan et al 1995) and evidence for higher prevalence of type 2 diabetes in mothers of women with GDM (Martin et al 1985). HLA DR3 and DR4 antigens are in higher frequency in women with GDM than in women with normal pregnancies. Furthermore, an association between variation in the insulin receptor (*INSR*) in Caucasian and African-American women with GDM has been reported (Ober et al 1989). A β-cell defect is one of the primary characteristics of GDM and β-cell function is a highly heritable trait(Watanabe et al 2007).

4. Association between GDM and oxidative stress and diabetic complications

Pregnancy is susceptible to oxidative stress and antioxidant defenses that can be altered in response to elevated levels of oxidative stress (Chen and Scholl 2005. Marfella et al 2001, Maxwell et al 1997).

In GDM products of lipid peroxidation may be increased and antioxidant enzyme activities decreased and the oxygen free radicals may be involved in severe damage of cellular structure (Osawa andKato 2005, Twardowska-Saucha et al 1994) and pregnancy complicated by poor glycemic control is associated with a higher risk of embryopathies, spontaneous abortion and perinatal morbidity and mortality (Loeken 2006). Recently, Karacay et al (2010) demonstrated that plasma and serum maternal total antioxidant status (TAS) was decreased, while circulating levels of lipid peroxidation breakdown products (MDA) were increased between 24 and 36 weeks of gestation, thus showing that increased oxidative stress and reduction in antioxidant defense mechanisms may contribute to disease processes in GDM (Bertazzi et al 2001, Karacy et al 2010, Rustemeijer et al 2001). Carine et al (1993) and Zachara et al (1993) found no differences in glutathione peroxidase (GPX) levels between pregnant women at third trimester and non-pregnant women, but recent studies have demonstrated an association between GDM and impaired SOD activities and enhanced circulating lipid metabolite levels such as MDA (Grissa et al 2007). Catalase, the main regulator of hydrogen peroxide metabolism is involved in Glut 4 expression, insulin secretion, insulin signaling, protein tyrosine phosphatase regulation, and glucose transport stimulation (Goth et al 2005, Mueller et al 1997). Catalase is important in antioxidant defense against hydrogen peroxide and increased risk of diabetes has been reported in hereditary catalase deficiency (Goth and Eaton 2000, Sindhu et al 2004).

5. Biology of BuChE

The enzyme cholinesterase is present in all mammals and two classes have been identified :acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE); AChE exists in the central nervous system, platelets and the erythrocyte membrane, while BChE is more abundant in the serum and is synthesized by the liver (Daresh et al 2003). BuChE was

named "pseudocholinesterase" by Mendel and Rudney in 1943 (1943). Human plasma BuChE (EC 3.1.1.8) is a globular, tetrameric serine esterase with a molecular mass of ≈340 kDa that is stable in plasma with a half-life of 12 days (Lockridge et al 1987, Ostergaard et al 1988). BuChE acts on hydrophilic and hydrophobic choline esters, and that it hydrolyzes a variety of xenobiotics as shown in Table 2. Previous studies have reported a significant association between the serum BuChE activity and obesity, coronary artery disease, serum levels of triglycerides (TG), very low-density lipoprotein, low-density lipoprotein and Apo lipoprotein B, type 2 diabetes mellitus and the hepatic fat content (Alcantara et al 2005, Cucuianau et al 1999, Randell et 2005, Sridhar et al 2005). At variance with AChE-S, BuChE attenuates the fibril-formation process by the aromatic W8 residue. This residue can form heteroaromatic complexes with soluble monomeric or low-oligomeric Aβ conformers. That replacement of tryptophan to a polar residue abolishes the attenuation of Aβ fibril formation is fully compatible with this hypothesis. AChE mRNA is 20-fold more abundant than BuChE mRNA. In human blood, however, BuChE, at 50 nM, is 3-fold more abundant than AChE (Daresh et al 2003).

BuChE protein or mRNA has been found in almost every tissue of the body, showing that it has a function.
1. Acetylcholine and butyrylthiocholine hydrolysis.
2. Protection from neurotoxins
- OP nerve agents
- OP pesticides
- Carbamate pesticides
- Alzheimer drugs-donepezil and rivastigmine
- Physostigmine in the calabar beans
- Cocaine from Erythroxylum coca plant
- Solamidine from green potatoes
- Luperzine A from the club moss
- Anatoxina in the blue green algae.
3. Hydrolysis of short-acting muscle relaxants
- Succinylcholine
4. Not clear yet
- Glucose and lipid metabolism

Table 2. Functions of BuChE

5.1 Genetics of BuChE

The complete amino acid sequence of human serum BuChE have been described (Daresh et al 2003). The human butyrylcholinesterase (BuChE; EC 3.1.1.8) is encoded by a single gene which corresponds to the E1 locus *BuCHE* gene (3q26.1-q26.2) which presents four exons (Arpagaus et al 1993), with more than 70 already-described variants (Pantuck 1993, Souza et al 2005). Data from dizygotic twin pairs has shown linkage on chromosome 3 at the location of the BuChE gene and also on chromosome 5. BuChE is found in human plasma, either in homomeric viz. monomers (G1), dimers (G2), trimers (G3) and tetramers (G4), or heteromeric forms associated with other substances, such as albumin (G1-ALB) (Masson et

al 1989, Pantuck 1993). The *BCHE-K* variant has been reported to show allelic association with Alzheimer disease (AD) in subjects who are also carriers of the e4 allele of apolipoprotein E (*APOE)*, especially in subjects over the age of 75 years. The K variant, is carried on one allele by one of four persons (Rao et al 2006). As BuChE is found common to both Alzheimer's disease and diabetes; it may play an etiological role via influencing insulin resistance and lipid metabolism (Arpagaus et al 1990, Lockridge et al 1987, Rao et al 2006). Similarly patients with Alzheimer's disease are more vulnerable to developing impaired fasting glucose and type 2 diabetes mellitus (Janson et al 2004, Johansen et al 1991)

5.2 BuChE and placental development

In utero exposure to poisons and drugs (e.g., anticholinesterases, cocaine) is frequently associated with spontaneous absorption and placental malfunction. The major protein interacting with these compounds is butyrylcholinesterase (BuChE), which attenuates the effects of such xenobiotics by their hydrolysis or sequestration. Sternfeld and Associates (1997) studied BuChE expression during placental development. RT-PCR revealed both BuChE mRNA and acetylcholinesterase (AChE) mRNA throughout gestation. Maximum butyrylcholinesterase activity has shown in week 12. In rat placenta, BuChE activity on gestational day 21 reached 150% of the level on gestational day 16. BuChE detoxifies anticholinesterases (AC) and other toxins including free radicals that are known to threaten pregnancy (Hollander et al 2007, Maxwell et al 1997, Osawa and Kato 2005, Twardowska-Saucha et al 1994). There is evidence that Kuwaiti women experiencing disorders of pregnancy like preeclampsia and diabetes mellitus in pregnancy exhibited lower serum activity of BuChE (Mahmoud et al 2003, Mahmoud et al 2006, Mahmoud et al 2008).

6. Clinical role of BuChE

BuChE (BuChE; EC 3.1.1.1.8) has well-defined pharmacologic functions:
BuChE and anaesthetic muscle relaxants: Mivacurium and succinylcholine are short-acting neuromuscular blocking drugs ideal for short surgical procedures as muscle relaxants used in anesthetic practice. The brief duration of action depends on rapid hydrolysis by plasma cholinesterase (Jensen et al 1991Pantuck 1993). An inherited or acquired deficiency of plasma BuChE can prolong the effect of mivacurium. When there is a deficiency of this enzyme due to the presence of one or more atypical alleles, mivacurium and succinylcholine are not properly metabolized and thus muscle paralysis can last for several hours (Davis et al 1997, Goudsouzian et al 1993, Petersen et al 1993, Savarese et al 1997).

6.1 Factors affecting BuChE activity

Different disease states and/or drug administrations may decrease BuChE activity.; such as extremes of age, pregnancy, renal and liver disease, malignancy, burns, chronic debility/malnutrition, myocardial infarction/cardiac failure, collagen diseases, myxedema, poisoning and protein energy malnutrition. Drugs that inhibit the enzyme's activity include acetylcholinesterase inhibitors (neostigmine, pyridostigmine, physostigmine, and edrophonium), anticholinesterases (especially echothlophate), cytotoxic agents (such as cyclophosphamide), steroids, ester-type local anesthetics, hexafluorenium, pancuronium, oral contraceptives and sertraline (Klein-Schwatz and Anderson 1996, MacQueen et al 2001, Muller et al 2002).

6.2 BuChE and Organophosphatase (OP) and cocaine hydrolysis

Another reason for continued interest in serum cholinesterase is its extraordinary sensitivity to organophosphate ester. Systemic administration of BuChE, at a dose sufficient to increase plasma BuChE levels 400-fold (5000 I.U.; i.v.), has been shown to significantly decrease cocaine-induced locomotor activity in rats over a 120-min session (Carmona et al 1997). The identification of BuChE variants that exhibit increased cocaine hydrolysis activity provides treatment options for cocaine-induced conditions such as cocaine overdose and addiction (Arkhypova et al 2004, Lockridge et al 2005), Lynch et al 1997).

6.3 BuChE activity and dyslipidemia and metabolic syndrome

Serum levels of BuChE are affected by dietary fat, obesity, hyperlipidemia and diabetes mellitus, alcohol and many drugs are known to increase BuChE activity (Alcantara et al 2005, Stefanello et al 2005, Vrdoljaki et al 2005). Therefore, BuChE may have a role in the altered lipoprotein metabolism in hypertriglyceridaemia associated with diabetes mellitus and insulin resistance. BuChE is synthesized in the liver, and is present in plasma and to a lesser extent in adipose tissue, small intestine and smooth muscle. Sridhar et al (2005) measured the serum level of BuChE levels in persons with type 2 diabetes mellitus and demonstrated a negative correlation between BuChE and serum total cholesterol and LDL cholesterol, thus further confirming that BuChE may be involved in lipid metabolism.

6.4 Serum determination of BuChE

The application of the techniques of molecular genetics has permitted precise identification of plasma cholinesterase variants and has resulted in the discovery of previously unrecognized variants. Serum BuChE activity has been determined by the method of Ellman et al (1961). In addition to colorimetric methods, HPLC, Electrophoresis, Immunoassay methods (ELIZA) and Biosensor methods have been used.

6.5 Production of human BuChE

Human BuChE has been obtained from human plasma by a large scale purification technique (Lockridge et al 2005). This procedure is severely limited by the volume of human plasma needed and may not be cost effective and it may not yield a sufficient amount of enzyme purified commercially. Large quantities of BuChE are needed for effective prophylaxis and treatment of exposure. BuChE has a broad spectrum of activity, a relatively long half-life, and few physiological side effects. Producing recombinant BuChE (rBuChE) is an alternative to purification of the enzyme from human plasma. A number of studies have shown the feasibility of producing large quantities of BuChE in transgenic animals (goats) and transgenic edible plants for prophylaxis or treatment of humans exposed to OP agents (Lockridge et al 2005, Podoly et al 2008, Protexia 2011) and cocaine overdose or addiction (Om et al 1993).

7. Association between BuChE and oxidative stress

Stefanello et al. (2005) investigated the effect of homocysteine administration on BuChE activity in the serum of rats. Acute and chronic administration of homocysteine significantly decreased BuChE activity but administration of vitamins A and C prevented the reduction of the activity. Delwing et al (2005) observed that acute proline administration provoked a 22% increase in BuChE activity in the serum of rats. In a similar study, Wyse et al (2004)

demonstrated that vitamins E and C reversed the inhibition of BuChE activities provoked by arginine in the serum of rats, thus indicating that the reduction of BuChE activities caused by arginine was probably mediated by oxidative stress. In a similar fashion, Cederberg et al. (2001) have shown that combined treatment with vitamins E and C decreased oxidative stress and improved fetal outcome in experimental pregnancy. From the foregoing, BuChE may yet be another mechanism in the fight against oxidative stress.

7.1 Mechanisms of the association between BuChE and oxidative stress in GDM

In a recent report, we (Omu et al 2010) showed that BuChE activity was elevated in the serum and placenta in normal pregnancy as compared to diabetic cohorts ($p < 0.01$) and there was a higher activity level in gestational and type 2 diabetes on insulin ($p<0.05$) compared with diet controlled. Conversely, there was higher MDA and lower antioxidant activity in diet versus insulin controlled diabetes ($p < 0.01$). Both serum and placental BuChE activity showed a strong inverse correlation with MDA ($r = -0.876$, $p < 0.001$) and ($r = - 0.542$, $p < 0.01$), but strong positive correlation with total antioxidant activity in serum ($r = 0.764$, $p < 0.001$) and placenta ($r = 0.642$, $p < 0.01$). These results are therefore consistent with a mechanism in which BuChE acts to scavenge free radicals in the presence of oxidative stress. An interesting finding in the study was the higher BuChE activity in the two groups of insulin-treated diabetics compared with their counterparts on diet. This led to the speculation that the diabetic patients on diet only might not have had satisfactory glycogenic control. However, BuChE did not show any correlation with enzymatic antioxidants SOD and GPX (Omu et al 2010); indirectly showing that BuChE was not inhibiting MDA through the antioxidants pathway. While this is mere speculation, it has important clinical implication if the association between BuChE and glycemic control is confirmed by future research. HbA1c has been used for monitoring diabetic control of the last 3 months, maybe BuChE could be used for short term or immediate monitoring of glycemic control. BuChE is already a known marker of metabolic syndrome (Sridhar et al 2005), and its activity is high in human term placenta (Hahn et al 1993, Lappas et al 2010, Omu et al 2010, Simone et al 1994, Sternfield et al 1997). The lower level of placental BuChE activity compared with serum, shown in the study may be as a result of a high level of fetotoxic agents, including free radicals (oxidative stress), in the placenta that BuChE metabolises by hydrolysis.

7.2 Advanced glycation end-products (AGE), reactive oxygen species (ROS) and BuChE

Glycation reactions lead to the production of reactive oxygen species (ROS), which are harmful to cellular metabolism and cause cell damage. There are no research data of any relationship between AGE and BuChE activity. While it is highly speculative, BuChE may protect pregnancy from the effect of oxidative stress by preventing the (formation) of reactive oxygen species formation by hydrolyzing and inactivating advanced glycation end products upstream. This hypothesis is consistent with the finding of lack of correlation between BuChE and SOD and GPX (Omu et al 2010).

8. Gestational diabetes mellitus and BuChE

Another contributor to toxicant-induced immune dysregulation as a contributor to GDM might be that reactive products of inflammation expressed by the maternal immune system

in response to paternal antigens adversely affect maternal health in ways that increase susceptibility to diabetes, thereby leaving women with naturally lower BuChE levels at greater risk for gestational and possibly Type 2 diabetes. The relationship between size of activated lymphocyte cohorts and BuChE activity in the RPL versus healthy cohorts provides additional support for the hypothesis that both immune activation and BuChE activity may be tied to some as-yet-unidentified systemic effector. For example, our observation of positive correlation between the frequency of CD3+CD16+CD56+ cells and BuChE activity in healthy individuals but not RPL-afflicted subjects would be expected if these cells which are often pathogenic, were expanded in response to environmental toxins. In the type 2 diabetes mellitus population serum BuChE activity has been correlated with insulin sensitivity ($r = -0.51$, $P < 0.001$). BuChE activity was elevated in the serum and placenta in normal pregnancy versus diabetic cohorts ($p < 0.01$) and there was a higher activity level in gestational and type 2 diabetes on insulin ($p < 0.05$) compared with diet controlled (Omu et al 2010, Mahmoud et al 2003, Mahmoud et al 2006, Mahmoud et al 2008, Rustemeijer et al 2001)

8.1 BuChE and congenital anomalies

BuChE may also play a significant role in congenital anomalies. Dupont et al. (1995) have reported that fetuses with anencephaly and open spinal bifida and gastroschisis revealed clearly dense band of BuChE in the amniotic fluid. A causative role for elevated free fatty acid (FFA) levels in the development of microvascular complications remains to be established, however. Increased levels of FFAs are positively correlated with both insulin resistance and the deterioration of ß-cell function in the context of concomitant hyperglycemia. These latter effects may result from oxidative stress (Evans et al 2003).

9. Future directions and hypotheses of connection between BuChE and GDM

There is need to explore a number of hypotheses to fully unravel the connection between GDM and BuChE through aggressive research efforts.

9.1 Role of estrogen alpha receptor and BuChE in pathogenesis of gestational diabetes

High levels of estrogens in the second half of pregnancy with high estrogen receptor alpha (ER α) lead to deterioration of glucose metabolism. Estrogens may reduce the risk of AD through enhancing or preserving cholinergic neurotransmission, and aromatase, the product of the CYP19 gene, is a critical enzyme in the peripheral synthesis of estrogens. There is evidence to suggest that the CYP19 and BuChE polymorphisms may interact in determining the risk of AD. Carriers of both the ER-a P/P genotype and the BuChE K variant would have decreased risk of developing AD (Conbarros et al 2007). ER alpha signaling activity and glucose metabolism may therefore be affected by CYP19 and BuChE polymorphisms.

9.2 Advanced glycation end products hydrolysis by BuChE

Advanced glycation end products may inhibit BuChE activities, probably as a result of the hydrolyzing effect of the latter, upstream before they cause oxidative stress.

10. Concluding remarks

Unraveling the connection between GDM and BuChE has become a veritable area of research in the pathogenesis, screening, prevention and management. With the large scale purification of BuChE from human plasma, milk of transgenic goats and edible transgenic plants and its suitability for prophylactic and therapeutic protection against cocaine and nerve agent toxicity, the way for therapeutic use in humans, especially during complicated pregnancy needs urgent scientific exploration as BuChE may have an important protective role in normal and diabetic pregnancy by reducing oxidative stress and therefore reduce diabetes induced complications. Mechanisms for attenuation of the effects of oxidative stress by BuChE should be investigated. Heritable factors may be an underlying biological thread in the connection between GDM and BuChE. In addition, genetic variants of BuChE exist, which may play a role in biological manifestation of individuals. Identification of such sequences would provide leads for further understanding of aetiological, therapeutic or prognostic aspects of Gestational diabetes mellitus. If future studies reveal that immune dysregulation is a contributor to the pathogenesis of GD or DM, characterization of the mechanisms will open additional avenues to development of therapeutic approaches to both disorders.

11. References

Alcantara VM, Oliveira LC, Rea RR, Suplicy HL, Chautard-Freire-Maia EA. Butyrylcholinesterase activity and metabolic syndrome in obese patients. *Clin Chem Lab Med.* 2005;43:285–8.

American Diabetes Association. Gestational diabetes mellitus. *Diabetes Care*. 2003; 26: S103–S105.

Arkhypova VN.,. Dzyadevychlexey S V, Soldatkin P, Korpan Y I. El'skaya A V, Gravoueille J-M, Martelet C and Jaffrezic-Renault N. Application of enzyme field effect transistors for fast detection of total glycoalkaloids content in potatoes. *Sensors and Actuators B: Chemical* 2004; 103: 416-422

Arpagaus M, Kott M, Vatsis KP, Bartels CF, La Du BN and Lockridge O Structure of the gene for human butyrylcholinesterase. Evidence for a single copy. *Biochemistry* 1990; 29:24-131.

Baccarelli A, Mocarelli P, Patterson DG, Bonzini M, Pesatori AC, Landi MT: Immunologic effects of dioxin: new results from Seveso and comparison with other studies. *Environ Health Persp* 2002; 110:1169–1173.

Bertazzi PA, Consonni PA, Bachetti S et al. Health effedts of dioxin exposure: a 20 years mortality study. *Am J Epidemiol* 2001; 153: 1031-1044

Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; 414 :813 –820.

Carine D, Loverco G, Greko P, Capuno F, Scivaggi L. Lipid peroxidation products and antioxidant enzymes in red blood cells during normal and diabetic pregnancy. *Eur J Obstet Gynaecol Reprod Biol* 1993; 51: 103-109.2.

Carmona GN, Schindler CW, Shoaib M, Jufer R, Cone EJ, Goldberg SR, Greig NH, Yu QS and Gorelick DA. Attenuation of cocaine-induced locomotor activity by butyrylcholinesterase. *Exp Clin Psychopharmacol* 1998; 6:274-279.

Cederberg J, Siman CM, Eriksson UJ.. Combined treatment with vitamins E and C decrease oxidative stress and improves fetal outcome in experimental diabetic pregnancy. *Pediatric Research* 2001; 49: 755–762.

Chen X, Scholl TO. Oxidative stress: changes in pregnancy and with gestational diabetes mellitus. *Curr Diab Rep.* 2005; 5: 282-288.

Combarros O, Riancho JA, Arozamena J, Mateo I, Llorca J, Infante J et al. Interaction between estrogen receptor-alpha and butyrylcholinesterase genes modulates Alzheimer's disease risk. *J Neurol*. 2007; 254: 1290-1292.

Cucuianu M. Serum gamma glutamyltransferase and/or serum cholinesterase as markers of the metabolic syndrome. *Diabetes Care.* 1999;22:1381-1382.

Darvesh S, Hopkins D A. & Geula C. Neurobiology of butyrylcholinesterase *Nature Reviews Neuroscience* 2003; 4: 131–138.

Davis L, Britten JJ, Morgan M. Cholinesterase. Its significance in anaesthetic practice. *Anaesthesia.* 1997;52:714

Delwing D, Chiarani F, Wannmacher CMD, Wajner M, Wyse ATS. Effect of hyperprolinemia on acetylcholinesterase and butyrylcholinesterase activities in rat. *Amino Acids* 2005; .28:305–308.

Dupont M, Vallet B, Brun A, Boulot P, Demaille J. Scanning gel densitometry of amniotic fluid acetylcholinesterase and butyrylcholinesterase: quantification of 'faint-positive' bands in fetal malformations. *Biol Neonate.* 1995; 67: 244-247

Ellman GL, Courtney KD, Andres V, Featherstone RM, A new and rapid colorimetric determination of Acetycholinesterase activity. *Bioch Pharmacol* 1961; 7: 88-95

Elobeid, M.; Allison, D. "Putative environmental-endocrine disruptors and obesity: a review" *Current opinion in endocrinology, diabetes, and obesity* 2008; 15: 403–408.

Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, Furlong CE, Holland NT: Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environ Health Perspect* 2004; 112:1116–1124.

Evans J L, Goldfine I D, Maddux B A, and Grodsky G M.Are Oxidative Stress-Activated Signaling Pathways Mediators of Insulin Resistance and ß-Cell Dysfunction? *Diabetes* 2003; 52 :1-8.

Gabbe SG, Graves CR. Management of diabetes mellitus complicating pregnancy. *Obstet Gynecol* 2003; 102: 857–68.

Góth L, Eaton JW. Hereditary catalase deficiencies and increased risk of diabetes. *Lancet* 2000;356:1820-1821

Góth L, Tóth Z, Tarnai I, Bérces M, Török P and Bigler W N. Blood Catalase Activity in Gestational Diabetes Is Decreased but Not Associated with Pregnancy Complications *Clinical Chemistry.* 2005;51:2401-2404.

Goudsouzian NG, d'Hollander AA, Viby-Mogensen J. Prolonged neuromuscular block from mivacurium in two patients with cholinesterase deficiency. *Anesth Analg.* 1993;77:183–185.

Grissa O, Atègbo JM, Yessoufou A, Tabka Z, Miled A, Jerbi M, Dramane KL et al. Antioxidant status and circulating lipids are altered in human gestational diabetes and macrosomia. *Transl Res* 2007; 150:164-71.

Hahn T, Desoye G, Lang I, Skofitsch G. Location and activities of acetylcholinesterase and butyrylcholinesterase in the rat and human placenta. *Anat Embryol.* 1993;188: 435-40.

Hollander MH, Paarlberg KM, Huisjes AJ. Gestational diabetes: a review of the current literature and guidelines. *Obstet Gynecol Surv.* 2007;62:125–136.

HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008;358:1991–2002.

Janson J, Laedtke T, Parisi JE, Brien PO, Petersen RC, Butler PC: Increased risk of type 2 diabetes in Alzheimer disease. *Diabetes* 2004, 53:474-81].

Jensen FS, Viby-Morgensen J, Ostergaard D. Significance of plasma cholinesterase for the anaesthetist. *Curr Anaesth Crit Care.* 1991;2:232–237.

Johansen AE-M, Nielsen D, Andersen G, Hamid YH, Jensen DP, Glümer C, Drivsholm T, Borch-Johnsen K, Jørgensen T, Hansen T, Pedersen O: Large-scale studies of the functional K variant of the butyrylcholinesterase gene in relation to Type 2 diabetes and insulin secretion. *Diabetologia* 2004, 47:1437-41.

Karacay O, Sepici-Dincel A, Karcaaltincaba D, Sahin D, Yalvaç S, Akyol M, Kandemir O, Altan N. A quantitative evaluation of total antioxidant status and oxidative stress markers in preeclampsia and gestational diabetic patients in 24-36 weeks of gestation. *Diabetes Res Clin Pract.* 2010; 89: 231-8.

King GL, Loeken MR.Hyperglycemia-induced oxidative stress in diabetic complications. *Histochem Cell Biol.* 2004; 122: 333-8

Klein-Schwartz W, Anderson B. Analysis of sertraline-only overdoses. *Am J Emerg Med.* 1996;14:456–458.

Kyriakis, J.M., Banerjee, P. Nikolakaki, E., Dai, T., Rubie, E.A., Ahmad, M.F., Avruch, J. and Woodgett, J.R. The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* 1994, 369: 156-60.

Lappas M, Mitton A, Permezel M. Release of Proinflammatory Cytokines and 8-Isoprostane from Placenta, Adipose Tissue, and Skeletal Muscle from Normal Pregnant Women and Women with Gestational Diabetes Mellitus. *J Endocrinol. 2010; 204; 75-84.*

Lockridge O, Bartels CF, Vaughan TA, Wong CK, Norton SE, Johnson LL. Complete amino acid sequence of human serum cholinesterase. *J Biol Chem.* 1987;262:549–557.

Lockridge O, Schopfer LM, Winger G, Woods JH Large scale purification of butyrylcholinesterase from human plasma suitable for injection into monkeys; a potential new therapeutic for protection against cocaine and nerve toxicity. *Journal of Medical Chemical, Biological and Radiological Defense.* 2005; 3:1–20.

Loeken MR. Advances in understanding the molecular causes of diabetes-induced birth defects. *J Soc Gynecol Investig*. 2006;13: 2-10.

Lynch TJ, Mattes CE, Singh A, Bradley RM, Brady RO, Dretchen KL. Cocaine detoxification by human plasma butyrylcholinesterase. Toxicol Appl Pharmacol 1997;145:363–371

MacQueen G, Born L, Steiner M. The selective serotonin reuptake inhibitor sertraline: its profile and use in psychiatric disorders. *CNS Drug Rev.* 2001;7:1–24.

Mahmoud F, Haines D, Abul H, Omu A. Butyrylcholinesterase activity and pregnancy-associated differences in immunologically relevant peripheral blood leukocyte populations. . *Am J Reprod Immunol.* 2003;50:77-82.

Mahmoud FF, Haines DD, Abul HT, Omu AE, Abu-Donia MB. Butyrylcholinesterase activity in gestational diabetes: correlation with lymphocyte subpopulations in peripheral blood. *Am J Reprod Immunol.* 2006; 56:185-92.

Mahmoud FF, Abul HT, Haines DD, Omu AE, Diejomaoh M, Wise JA, Abu Donia MB. Butyrylcholinesterase activity and lymphocyte subpopulations in peripheral blood of Kuwaiti women experiencing recurrent spontaneous abortion. *Reprod Immunol.* 2008;77:186-94.

Marfella R, Quagliaro L, Nappo F, Ceriello A and Giugliano D. Acute hyperglycemia induces an oxidative stress in healthy subjects. J Clin Invest 2001; 108 : 635-636.

Martin AO, Simpson JL, Ober C, Freinkel N: Frequency of diabetes mellitus in mothers of probands with gestational diabetes: possible maternal influence on the predisposition to gestational diabetes. *Am J Obstet Gynecol* 1985; 151:471–475.

Masson P A naturally occurring molecular form of human plasma cholinesterase is an albumin conjugate. *Biochim Biophys Acta* 1989; 988:258-266.

Massoulie´ J, Bon S, Anselmet A, Chatel J-M, Coussen F, Duval N, Krejici E, Legay C, Vallette F.Biosynthesis of the molecular forms of acetylcholinesterase. In Multidisciplinary Approaches to Cholinesterase Functions, A Shafferman, B Velan (eds). New York, Plenum Press, 1992, pp 17–24.

Mauricio D, de Leiva A. Autoimmune gestational diabetes mellitus: a distinct clinical entity? *Diabetes Metab Res Rev* 2001;17 : 422-2

Maxwell SRJ, Thomason H, Sandler D, Leguen C, Baxter MA, Thorpe GHG, Jones AF, Barnett AH Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. *Eur J Clin Invest* 1997; 27:484–490

McLellan JA, Barrow BA, Levy JC, Hammersley MS, Hattersley AT, Gillmer MD, Turner RC: Prevalence of diabetes mellitus and impaired glucose tolerance in parents of women with gestational diabetes. *Diabetologia* 1995;38: 693–698

Mendel B. and Rudney H. Cholinesterase and pseudo-cholinesterase. *Biochem. J.* 1943; 37: 59-63.

Metzger BE, Coustan DR, the Organizing Committee. Summary and recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes Care* 1998; 21: B161–167

Morgan SC, Relaix F, Sandell LL, Loeken MR. Oxidative stress during diabetic pregnancy disrupts cardiac neural crest migration and causes outflow tract defects. *Birth Defects Res A Clin Mol Teratol.* 2008; 82: 453-63.

Mueller S, Riedel HD, Stremmer W. Direct evidence for catalase as the predominant H_2O_2 removing enzyme in human erythrocytes. *Blood* 1997; 90: 4973-4978.

Müller TC, Rocha JB, Morsch VM, Neis RT, Schetinger MR. Antidepressants inhibit human acetylcholinesterase and butyrylcholinesterase activity. *Biochim Biophys Acta.* 2002;1587:92–98

Nadal A, Alonso-Magdalena P, Soriano S, Quesada I, Ropero AB. The pancreatic beta-cell as a target of estrogens and xenoestrogens: Implications for blood glucose homeostasis and diabetes. *Mol Cell Endocrinol.* 2009; 304: 63-8

National Institute for Health and Clinical Excellence. NICE clinical guideline 63: *Diabetes in pregnancy.Management of diabetes and its complications from pre-conception to the postnatal period.* London: NICE; 2008

Newbold R.; Padilla-Banks E.; Jefferson W. "Environmental estrogens and obesity". *Molecular and cellular endocrinology* 2009; 304: 84–89.

Ober C, Xiang KS, Thisted RA, Indovina KA, Wason CJ, Dooley S: Increased risk for gestational diabetes mellitus associated with insulin receptor and insulin-like growth factor II restriction fragment length polymorphisms. *Genet Epidemiol* 1989; 6:559–56.

Om A, Ellahham S, Ornato JP, Picone C, Theogaraj J, Corretjer GP, Vetrovec GW. Medical complications of cocaine: Possible relationship to low plasma cholinesterase enzyme. Am Heart J 1993; 125:1114–1117

Omu AE, Al-Azemi MK, Omu FE, Fatinikun T, Abraham S, George S, Mahnazhath N. Butyrylcholinesterase activity in women with diabetes mellitus in pregnancy: correlation with antioxidant activity. *J Obstet Gynaecol.* 2010; 30:122-6.

Osawa T, Kato Y. Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. *Ann N Y Acad Sci.* 2005; 1043:440-51.

Ostergaard D, Viby-Mogensen J, Hanel HK, Skovgaard LT. . Halflife of plasma cholinesterase. *Acta Anaesthesiol Scand.* 1988; 32:266–2696.

Paloma A M, Ropero A B, Carrera M. P,. Cederroth C R, Baquié M, Gauthier B R., Nef S, Stefani E and Nadal A. Pancreatic Insulin Content Regulation by the Estrogen Receptor ERα. PLoS ONE. 2008; 3: e2069.

Pantuck EJ. Plasma cholinesterase: gene and variations. Anesth Analg. 1993 ;77:380-86.

Petersen RS, Bailey PL, Kalameghan R, Ashwood ER. Prolonged neuromuscular block after mivacurium. *Anesth Analg.* 1993;76:194–196.

Podoly E, Bruck T, Diamant S, Melamed-Book N, Weiss A, Huang Y, Livnah O, Langermann S, Wilgus H, Soreq. Human recombinant butyrylcholinesterase purified from the milk of transgenic goats interacts with beta-amyloid fibrils and suppresses their formation in vitro. *Neurodegener Dis.* 2008;5: 232-6.

Protexia -Gene-Delivered Butyrylcholinesterase Is Prophylactic against the Toxicity of Chemical Warfare Nerve Agents and Organophosphorus Compounds *J. Pharmacol. Exp. Ther.* 2011: 337; 92-101

Randell EW, Mathews MS, Zhang H, Seraj JS, Sun G. Relationship between serum butyrylcholinesterase and the metabolic syndrome. *Clin Biochem.* 2005;38:799–805.

Rao A A, Sridhar G R, Thota H, Babu C S, Prasad A S, Divakar C. Alzheimer's disease and Type 2 diabetes mellitus: the cholinesterase connection? *Lipids in Health and Disease* 2006, 5:28-34.

RCOG Scientific Advisory Committee Opinion Paper 23: Diagnosis and Treatment of Gestational Diabetes. January 2011.

Remillard RBJ, Bunce NJ. Linking Dioxins to Diabetes: Epidemiology and Biologic Plausibility. *Environ Health Perspect* 2002; 110:853-858.

Ropero AB, Alonso-Magdalena P, Quesada I, Nadal A. The role of estrogen receptors in the control of energy and glucose homeostasis. *Steroids*.2008.; 73; 874-79.

Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L: The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association, and the German Diabetes Society. *Diabetes Metab Res Rev* 2001;17 :189 – 212,

Rubin B, Soto A. "Bisphenol A: Perinatal exposure and body weight". *Molecular and cellular endocrinology* 2009; 304: 55–62.

Rustemeijer C, Schouten JA, Voerman HJ, Beynen AC, Donker AJM, Heines, RJ. Is pseudocholinesterase activity related to markers of triacylglycerol synthesis in type II diabetes mellitus? *Clin Sci* 2001;101:29-35

Saito S, Sasaki Y, Sakai M. CD4(+)CD25 high regulatory T cells in human pregnancy. *J Reprod Immunol.* 2005; 65:111–120.

Savarese JJ, Lien CA, Belmont MR, Wastila WB. The clinical pharmacology of new benzylisoquinoline-diester compounds. with special consideration of cisatracurium and mivacurium. *Anesthetist.* 1997;46:840–849

Serlin DC and. Lash, R W. Diagnosis and Management of Gestational Diabetes Mellitus. *Am Fam Physician.* 2009 ; 80: 57-62.

Simone C, Derewlany LO, OskampM, Johnson D, Knie B, Koren GAcetylcholinesterase and butyrylcholinesterase activity in the human term placenta: implications for fetal cocaine exposure. *Journal of Laboratory and Clinical Medicine*. 1994. 123: 400–406

Sindhu RK, Koo JR, Roberts CK, Vazioori ND. Dysregulation of hepatic superoxide dismutase, catalase, and glutathione peroxidase in diabetes: responses to insulin and antioxidant therapies. *Clin Exp Hypertens* 2004;26:43-53

Souza RLR, Mikami LR, Maegawa ROB and Chautard-Freire-Maia EA Four new mutations in the *BCHE* gene of butyrylcholinesterase in a Brazilian blood donor sample. *Mol Genet Metab* 2005; 84:349-353.

Sridhar GR, Nirmala G, Apparao A, Madhavi AS, Sreelatha S, Rani J S and Vijayalakshmi P Serum butyrylcholinesterase in type 2 diabetes mellitus: a biochemical and bioinformatics approach. *Lipids Health Dis.* 2005; 4: 18-.

Stefanello FM, Franzon R, Tagliari B, Wannmacher C, Wajner M, Wyse AT. Reduction of butyrylcholinesterase activity in rat serum subjected to Hyperhomocysteinemia. Metabolic Brain Disease 2005; 20:97–103.

Sternfeld M, Ben-Ari S, Rachmilewitz J, Glick C, Loewenstein-Lichtenstein Y, Soreq H, Andres C, Zakut H, Timberg R: Normal and atypical butyrylcholinesterases in placental development, function, and malfunction. *Cell & Mol Neurobiol* 1997; 17:315–332.

Twardowska-Saucha K, Grzeszczak W, Lacka B, Frehlich J, Krywult D. Lipid peroxidation, antioxidant enzyme activity and trace element concentration in II and III trimesters of pregnancy in pregnant women with diabetes. *Poskie Archiwum Medycyny Wewnetrzne j.* 1994; .92: 313-21.

Vrdoljaki A L, Bradamante V, Radi B, Peraica M, Fuchs R, Reine E. Butyrylcholinesterase activity and plasma lipids in dexamethasone treated rats *Acta Pharm* 2005; 55: 177–185.

Watanabe R M, Black M H, Xiang A H., Allayee H, Lawrence J M., Buchanan T A. Genetics of Gestational Diabetes Mellitus and Type 2 Diabetes. *Diabetes Care* 2007; 30: S134-S140

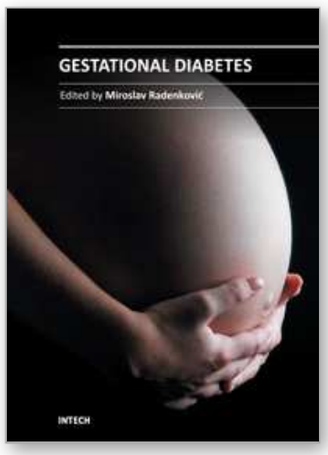
Wender-Ozegowska E, Kozlik J, Biczysko R, Ozegowski S. Changes of Oxidative Stress Parameters in Diabetic Pregnancy. *Free Radical Research,* 2004; 38 : 795-803.

World Health Organization. *Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1. Diagnosis and classification of diabetes mellitus.* Geneva: WHO, 1999; WHP/NCD/NCS/99.26p.

Wyse ATS, Stefanello FM, Chiarani F, Delwing D, Wannmacher CMD, Wajner M. Arginine administration decreases cerebral cortex acetylcholinesterase and serum butyrylcholinesterase by oxidative stress induction. *Neurochemical Research 2004; .29:385–389.*

Yogev, Y. & Visser, G. Obesity, gestational diabetes and pregnancy outcome. Seminars in Fetal & Neonatal Medicine 2009, 14, 77-84

Zachara BA, Wardak C, Didkowski W, Maciage A, Marchaluk E. Changes in blood selenium and glutathione peroxidase concentrations and glutathione peroxidase activity in human pregnancy. *Gynecol Obstet Invest* 1993; 35: 12-17.



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Gestational diabetes mellitus is defined as hyperglycemia with onset or first recognition during pregnancy. The incidence of gestational diabetes is still increasing and this pathological condition has strong association with adverse pregnancy outcomes. Since gestational diabetes can have long-term pathological consequences for both mother and the child, it is important that it is promptly recognized and adequately managed. Treatment of gestational diabetes is aimed to maintain euglycemia and it should involve regular glucose monitoring, dietary modifications, life style changes, appropriate physical activity, and when necessary, pharmacotherapy. Adequate glycemic control throughout the pregnancy can notably reduce the occurrence of specific adverse perinatal and maternal outcomes. In a long-term prospect, in order to prevent development of diabetes later in life, as well to avoid associated complications, an adequate education on lifestyle modifications should start in pregnancy and continue postpartum.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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