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EDITORIAL

Hypothesis: Intensive insulin therapy-induced mortality is due to excessive serotonin autoinhibition and autonomic dysregulation

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Abstract

Action to Control Cardiovascular Risk in Diabetes (ACCORD), The Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation and the Veterans Affairs Diabetes Trial were designed to study whether older patients with type 2 diabetes mellitus could reduce the risk of heart attacks and stroke and thereby prolong their lives by maintaining their blood glucose levels at near-healthy levels but failed to demonstrate the hoped-for benefit. Why the trials failed, though, and why ACCORD saw significantly more deaths due to increased rates of cardiovascular events in the intensive therapy arm of the study are not clear. These data have now been confirmed by the results of the recently concluded NICE-SUGAR Study which again revealed that intensive glucose control increased mortality among adults in intensive care units. I propose that the negative results noted in these trials are due to altered brain serotonin concentrations and autonomic dysregulation in addition to the low-grade systemic inflammation, decreased endothelial nitric oxide and enhanced free radical generation, diminished anti-oxidant defenses and altered metabolism of essential fatty acids present in patients with type 2 diabetes.

Key words: Type 2 diabetes mellitus; Cardiovascular events; Coronary heart disease; Stroke; Dyslipidemia; Essential fatty acids; Nitric oxide; Free radicals; Anti-oxidants; Insulin

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INTRODUCTION

It is believed that tight glycemic control (serum glucose 80-110 mg/dL) in critically ill adult patients and those with diabetes mellitus would prevent secondary complications due to diabetes and reduce morbidity and mortality. These recommendations are primarily based on one large trial in surgical patients[1] whereas other studies have shown lesser or no efficacy[2-7]. Wiener *et al*[2] sought to determine the efficacy and safety of tight glycemic control in critically ill adult patients by analyzing 29 published trials that included 8432 critically ill adult patients and found no

difference in rate of mortality or renal failure. There was an approximate 25% reduction in hospital-acquired sepsis but a 5-fold increased risk for hypoglycemia. On the basis of these results, it was concluded that tight glycemic control does not confer benefit for broad populations of critically ill adults and is associated with a greater risk for hypoglycemia. This meta-analysis supports the contention that tight glycemic control is not an appropriate strategy in critically ill adults. Surgical patients may derive benefit from such a strategy due to a combined infusion of glucose and insulin. The same benefit almost certainly does not exist for nonsurgical patients; in fact, tight glycemic control may cause more harm than benefit.

ACTION TO CONTROL CARDIOVASCULAR RISK IN DIABETES, ACTION IN DIABETES AND VASCULAR DISEASE: PRETERAX AND DIAMICRON MODIFIED RELEASE CONTROLLED EVALUATION, VETERANS AFFAIRS DIABETES TRIAL AND NICE-SUGAR STUDIES

The possibility that tight glycemic control may be harmful rather than beneficial is confirmed by the three recently concluded trials: Action to Control Cardiovascular Risk in Diabetes (ACCORD), The Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) and the Veterans Affairs Diabetes Trial (VADT)[8-11] that were designed to study whether older patients with type 2 diabetes mellitus could reduce the risk of heart attacks and stroke and thereby prolong their lives by maintaining their blood glucose levels at near-healthy levels but failed to demonstrate the hoped-for benefit. Why the trials failed, though, and why ACCORD saw significantly more deaths in the intensive therapy arm of the study in contrast to the expectation that chronic hyperglycemia in type 2 diabetes mellitus is associated with increased rates of cardiovascular events, is not clear. These results have now been confirmed by the NICE-SUGAR Study that showed that intensive glucose control increased mortality among adults in the intensive care units (ICU)[12]. But, two important facts need to be noted while drawing conclusions based on the ACCORD, ADVANCE, VADT and NICE-SUGAR Studies: ACCORD, ADVANCE and VADT studies were primarily conducted in subjects with type 2 diabetes mellitus and these patients also received oral hypoglycemic agents in addition to insulin (ACCORD: on insulin at study end 77 *vs* 55 in intensive *vs* standard therapy; ADVANCE: 40 *vs* 24 in intensive *vs* standard; VADT: 89 *vs* 74 in intensive *vs* standard respectively; whereas at study end 91 *vs* 58, 17 *vs* 11 and 53 *vs* 42 in ACCORD, ADVANCE and VADT studies respectively received thiazolidinediones)[11] whereas the NICE-SUGAR Study was in patients who were critically ill and received only insulin for the control of hyperglycemia.

TIGHTLY CONTROLLED BLOOD GLUCOSE IS BENEFICIAL IN TYPE 1 DIABETES MELLITUS

The Diabetes Control and Complications Trial (DCCT)[13] conducted in type 1 diabetes showed that keeping blood glucose levels as close to normal as possible slows the onset and progression of the eye, kidney and nerve damage caused by diabetes. When the DCCT ended in 1993, more than 90 percent of participants participated in the follow-up study called Epidemiology of Diabetes Interventions and Complications (EDIC)[14] which showed that intensive blood glucose control reduces the risk of any cardiovascular disease event by 42% and the nonfatal heart attack, stroke or death from cardiovascular causes by 57%.

DCCT AND EDIC *VS* ACCORD, ADVANCE AND VADT

Based on the results of the DCCT and EDIC studies and because the microvascular disease development process is likely to be similar for both type 1 and type 2 diabetes, it was assumed that intensive control of blood glucose will be beneficial even to those with type 2 diabetes. The United Kingdom Prospective Diabetes Study (UKPDS), a study involving patients with type 2 diabetes, demonstrated that controlling blood glucose levels reduced the risk of diabetic eye disease and kidney disease[15]. In contrast, the results of the ACCORD, ADVANCE and VADT trials showed that tight glycemic control by intensive therapy may be more harmful than beneficial.

Statistically, only 20% of NICE-SUGAR subjects had previously known diabetes compared to the outpatient studies with all type 2 diabetes in ACCORD, ADVANCE and VADT. The outpatient studies generally consisted of patients with long-standing, advanced diabetes, many of whom had complications and probably already had well-entrenched coronary artery disease. These patients are likely to already be at higher risk of cardiovascular events as well as autonomic dysfunction. It appears that patients with recently diagnosed diabetes without baseline macrovascular disease are better subjects for intensive control as suggested in subgroup analysis of ACCORD, ADVANCE and VADT. In comparison, DCCT and UKPDS were all newly diagnosed patients and long-term follow-up of patients originally assigned to the intervention group (albeit more relaxed targets than ACCORD *etc*) did demonstrate a reduction in cardiovascular events. Thus, the acuity of the intervention with respect to baseline risk and the duration of follow-up might be more important. It is assumed that most of the NICE-SUGAR patients had acute (stress) hyperglycemia and were unlikely to have developed severe autonomic neuropathy that is observed in patients with

advanced long-standing diabetes as in ACCORD. But, it may be mentioned here that both stress hyperglycemia and type 2 diabetes mellitus could cause an increase in norepinephrine and epinephrine and decrease in serotonin and its metabolites[16-23] in the brain and increased production and release of catecholamines from the phagocytes in the peripheral circulation. Furthermore, hypoglycemia (more common in those who were on intensive insulin therapy) is a potent stimulant of sympathetic nervous system that leads to production and release of catecholamines that, in turn, could enhance inflammatory injury in addition to their ability to cause life threatening tachyarrhythmias. Thus, subjects in ACCORD, ADVANCE, VADT and NICE-SUGAR studies would have had or developed severe autonomic disturbances either as a result of long-standing diabetes or as a result of acute hyperglycemia and hypoglycemia cycles-induced autonomic imbalance.

INTENSIVE INSULIN THERAPY OF HYPERGLYCEMIA IN ACUTELY ILL PATIENTS INCREASED MORTALITY

Hyperglycemia is common in acutely ill patients[24]. The occurrence of hyperglycemia, in particular, severe hyperglycemia, is associated with increased morbidity and mortality in a variety of groups of patients[25,26]. This is somewhat similar to uncontrolled hyperglycemia in patients with type 1 and type 2 diabetes mellitus. In view of this, it was assumed that tighter glucose control in acutely ill patients in intensive care units would be beneficial despite the fact that they were not diabetics to start with. But several trials examining the effects of tighter glucose control showed conflicting results[4-12,27,28] and the recently concluded NICE-SUGAR study found that intensive glucose control increased mortality among adults in the ICU[12]. These results are similar to those seen in ACCORD, ADVANCE and VADT studies. While discussing the differences among various studies[1,4-6,12,29], it was opined that insulin itself could have direct effects such as sympathetic activation, sodium retention or mitogenic actions that could have contributed to increased mortality seen[30]. Another possibility that needs to be considered is the neuroglycopenia that could occur as a result of hypoglycemia in these patients. In summary, intensive glycemic control approaching normoglycemia has not shown cardiovascular benefit, particularly in patients with advanced disease.

Since stress hyperglycemia in the critically ill is accompanied by insulin resistance and high plasma glucose levels, it is likely that these patients also show similar changes in the brain that have been described in type 2 diabetes mellitus: increase in norepinephrine and epinephrine and decrease in serotonin and its metabolites[16-23].

WHY IS INTENSIVE INSULIN THERAPY HARMFUL?

It is likely that patients with type 2 diabetes may have other biochemical abnormalities that could have skewed the results of ACCORD, ADVACE, VADT and NICE-SUGAR studies. For instance, they have low-grade systemic inflammation, decreased endothelial nitric oxide (eNO) production, enhanced plasma concentrations of asymmetrical dimethylarginine (ADMA) and free radical generation, diminished anti-oxidant defenses and altered metabolism of essential fatty acids (EFAs)[16-19]. It is possible that persistent hyperglycemia and intensive treatment could have depleted endothelial cells of their limited stores of L-arginine and EFAs that leads to gross deficiency of eNO and EFAs and their metabolites which could have enhanced their risk of cardiovascular events and stroke. This implies that subjects receiving intensive insulin therapy need co-administration of adequate amounts of L-arginine and EFAs and other co-factors for optimal formation of eNO and products of EFA metabolism (especially their anti-inflammatory compounds such as lipoxins, resolvins, protectins, maresins and nitrolipids) to prevent adverse cardiovascular events and stroke. Previously, we showed that oral administration of L-arginine enhanced eNO generation, increased insulin production and improved glycemic control[31]. Diabetics have decreased eNO[32] and increased amounts of plasma asymmetrical dimethylarginine (ADMA) that suppresses eNO synthesis and produces vascular dysfunction[33]. Supplementation of L-arginine lowers plasma ADMA levels and improves vascular dysfunction[31,34]. A close interaction exists between the L-arginine-NO system and EFAs in diabetes mellitus that could play an important role in the prevention of diabetes and restoring normal pancreatic β cell function[35-39].

INTENSIVE INSULIN THERAPY MAY ACTIVATE LEUKOCYTES AND MACROPHAGES AND ENHANCE INFLAMMATORY INJURY

Both obesity and type 2 diabetes mellitus are low-grade systemic inflammatory conditions[40,41]. Leukocytes and macrophages are the principal mediators of inflammation. Phagocytes release catecholamines during inflammation and catecholamines, in turn, enhance the inflammatory response[42]. Cross-talk between the autonomic nervous and the immune systems *via* sympathetic and parasympathetic pathways are known to exist. Vagal parasympathetic signalling suppresses inflammation through cholinergic receptors on phagocytic cells[43,44]. Thus, the functional interplay of the vagal parasympathetic nervous system with the immune/inflammatory system will counterbalance the effects of the adrenergic nervous system. These data suggest that phagocytes are a source of pro-inflammatory catecholamines and their enhanced or decreased production increases or decrease the intensity of inflammatory injury. This action is in addition to their ability to induce tachycardia and coronary ischemia.

Thus, patients with stress hyperglycemia and type 2 diabetes mellitus have an increase in norepinephrine and epinephrine and decrease in serotonin and its meta-

bolites[16-23] in the brain and may show increased production and release of catecholamines from the phagocytes in the peripheral circulation that could lead to an enhancement of inflammation in these subjects. The incidence of hypoglycemia is common when intensive insulin therapy is employed to control hyperglycemia in the acutely ill and patients with type 2 diabetes mellitus. Hypoglycemia is a potent stimulant of the sympathetic nervous system that incites an increase in the production and release of catecholamines that, in turn, enhances inflammatory injury in addition to their ability to cause life threatening tachyarrhythmias. Under normal conditions, a balance is maintained between the sympathetic and parasympathetic nervous systems. But in type 2 diabetics and the acutely ill, an increase in the production and release of catecholamines secondary to hypoglycemia and as a result of abnormalities in the catecholamines and serotonin in the brain would occur[16-23]. This may lead to suppression of the anti-inflammatory cholinergic parasympathetic system[43,44] resulting in further enhancement of inflammation.

MISMATCH BETWEEN PLASMA AND BRAIN INSULIN LEVELS CAUSES SYMPATHOVAGAL IMBALANCE IN TYPE 2 DIABETES MELLITUS AND STRESS HYPERGLYCEMIA

Fasting, hypoglycemia and streptozotocin-induced diabetes have been shown to influence brain tryptophan and serotonin metabolism[16-18]. Serum norepinephrine and epinephrine increased following injection of insulin in both the ventromedial and lateral hypothalamus with peaks at 60-90 min but serotonin was unchanged and its metabolite, 5-hydroxy-3-indole acetic acid (5-HIAA), fell gradually in both ventromedial and lateral hypothalamic areas. All monoamines and their metabolites reverted to normal within 30-60 min after the initiation of food intake[19], suggesting that hypoglycemia increases turnover of norepinephrine and serotonin.

In the alloxan-induced diabetic animal model, an increase in norepinephrine level in the anterior and the medial-basal hypothalamus and a concomitant rise in dopamine content in the hypothalamus was reported. In contrast, serotonin level fell in all the parts of hypothalamus in prediabetic animals[20].

Baranov *et al*[20] reported a decrease in serotonin level in the hypothalamus whereas Lackovic *et al*[25] showed that the contents of serotonin metabolites, i.e., 5-hydroxyindoleacetic acid and homovanillic acid, in the whole brain gradually decreased with the duration of diabetes.

One of the main functions of insulin is to regulate glucose homeostasis. Hence, it is likely that insulin and insulin receptors present in the brain modulate glucose metabolism not only in the brain but also in the peripheral tissues.

Intracerebroventricularly (icv) administered insulin in doses (50 and 100 μ Units) which induced minimal hypoglycemia, dopamine in midbrain-diencephalon and caudate nucleus (CN), norepinephrine and serotonin in midbrain and pons-medulla (PM) were more in the hyperglycaemic rats as compared to their euglycaemic counterparts whereas those of acetylcholine were lower in these three areas. Insulin induced a decrease in rat brain dopamine and norepinephrine levels which was more marked in the hyperglycaemic animals; and an insignificant increase in brain serotonin concentration. Insulin induced a marked increase in rat brain acetylcholine levels which was accentuated in hyperglycaemic animals. These studies suggest that an interaction exists among brain insulin receptors and monoamines and acetylcholine in euglycaemic and hyperglycaemic states[22] and that, possibly, insulin brings about some of its actions by modulating the levels of various monoamines in the brain.

In this context, it is noteworthy that although both in the lean and obese similar changes were found in the levels of insulin and monoamines, the responses seen in the obese were comparatively defective[16-19]: (1) Obese showed a dramatic increase in serotonin, 5-HIAA and dopamine at the beginning of spontaneous meals suggesting that they need a much higher stimulus for the feeling of satiety at the VMH-PVN level; (2) Glucoprivic feeding or satiety could be induced in normal rats by intravenous infusions of insulin or insulin + glucose respectively whereas obese were resistant to these treatments; (3) During meals, obese showed a much higher monoaminergic changes resembling those related to spontaneous feeding; (4) When meals were presented for the first time, VMH-PVN immunoreactive insulin increased earlier and with a smaller magnitude in the obese; and (5) In response to an intravenous insulin infusion, immunoreactive insulin in the brain increased twice as much in obese rats despite lower basal levels compared to the lean rats that suggests that, despite being insulin resistant, the obese Zucker rat have an inefficiency of the peptide in reducing feeding and body weight.

These results emphasize the fact that in obese rats and probably diabetic animals (and humans), the responses of the monoamines in the hypothalamus are inadequate both to the feeding and given plasma and brain insulin levels. This resistance to insulin results in the inappropriate res ponse seen in the monoamines. Hence, obese and possibly, type 2 diabetes animals (and humans) need supraphy siological increases in plasma glucose, insulin and monoa mines to signal the satiety feeling. In addition, the mismatch between plasma and brain insulin and hypothalamic monoamines suggests that the cross-talk between these two systems is defective in the obese and type 2 diabetes mellitus that may result in inappropriate inflammatory response both in the brain and peripheral tissues.

In addition, there are some very specific changes in the monoaminergic innervations of the central nervous system both in obesity and type 2 diabetes mellitus[23]. The monoaminergic innervations of the central nervous sys

tem are characterized by long and short projecting neu rons. It was reported that the long serotoninergic axons innervating the spinal cord and the cerebral cortex were unaffected in diabetic animals and that the noradren ergic innervations of the cortex was normal as well. The serotonin content was higher (almost twice as high) in the hypothalamus with no change in 5-HIAA levels, sugges ting supernumerary innervation that is accompanied by a reduced release. In the pons medulla oblongata, serotonin, dopamine and the metabolites 5-HIAA and DOPAC were significantly reduced whereas norepineph rine was markedly increased. In the hippocampus, there was a reduction of serotonin content. The distal projections of serotonin were normal accompanied by hyperinnervation of the hypothalamus but the shorter collaterals were lost in the pons medulla oblongata. It has been suggested that these alterations are due to lack/deficiency of insulin which could have triggered these monoaminergic alterations in the diabetic brain. These changes in the monoaminergic nerves coupled with the observation that there are some very specific interactions between insulin and hypothalamic monoamines indicate that insulin secretion, action and insulin responses in type 2 diabetes could be inappropriate.

Heart rate variability (HRV) can be used to estimate autonomic nervous control of the cardiovascular system. In middle-aged subjects, the metabolic syndrome (of which type 2 diabetes mellitus is an important component) is associated with lower HRV. In a study of 1889 subjects aged 24-39 years, it was noted that the presence of the metabolic syndrome was associated with lower HRV. Waist circumference was the strongest individual metabolic syndrome component that was associated with lower HRV, suggesting that, much before the development of frank type 2 diabetes mellitus, sympathovagal imbalance could exist. These results indicate that lower HRV could occur in young adults much before the development of type 2 diabetes mellitus and are consistent with lower vagal activity and an increase in sympathetic predominance in subjects with metabolic syndrome[45]. This decrease in vagal activity and sympathovagal balance may lead to inappropriate inflammation and the greater increase in cardiovascular risk seen in metabolic syndrome, type 2 diabetes mellitus and stress hyperglycemia (see Figure 1).

EXCESSIVE AUTOINHIBITION COULD RESULT IN AUTONOMIC DYSREGULATION AND SUDDEN DEATH

Thus, stress hyperglycemia and type 2 diabetes mellitus could cause an increase in norepinephrine and epinephrine level; a concomitant rise in dopamine content; a fall in serotonin level in the hypothalamus and other areas of the brain; and insulin, hypoglycemia and hyperglycemia may trigger inappropriate autonomic and serotonergic responses. Recent studies showed that a deficit in serotonin function is a risk factor for sudden infant death syndrome (SIDS). In these infants, immunohistochemical studies revealed an increased number of serotonin neurons as well as an increase in the fraction of serotonin neurons showing an immature, granular cell morphology suggesting a failure or delay in the maturation of these neurons. A significant decrease in serotonin receptor 1A Htr1a should be replaced by (Htr1a) and a decrease in relative serotonin transporter binding density was also noted in SIDS. These abnormalities would ultimately lead to serotonin deficits in SIDS and similar, if not identical, abnormalities in serotonin function appear to occur in obesity and type 2 diabetes mellitus[16-23]. Investigation into the consequences of altering the autoinhibitory capacity of serotonin neurons with the reversible overexpression of serotonin 1A autoreceptors in transgenic mice revealed that overexpressing mice exhibited sporadic bradycardia and hypothermia and frequently progressed to death[46,47]. This study[46] revealed that altered serotonin homeostasis alone is sufficient to precipitate catastrophic autonomic failure and death, a situation that seems to exist in obesity, type 2 diabetes mellitus and those who are critically ill.

TESTING THE HYPOTHESIS

It is possible that when patients with type 2 diabetes me llitus and the critically ill undergo intensive insulin therapy, it will lead to dramatic alterations in the brain and peripheral monoamines, serotonin and acetylcholine levels that could lead to sporadic autonomic dysregulation, excessive serotonin inhibition and inappropriate inflammation. These events could precipitate pronounced bra dycardia, apnea and death. It is possible that these pati ents may harbor functionally equivalent deficits in serotonin homeostasis that include alterations in local serotonin release, changes in intrinsic electrophysiological properties of serotonin receptors and deficiencies in auto regulatory feedback networks such as those involving norepinephrine. Hypoglycemic attacks that occur due to intensive insulin therapy, may, in turn, trigger sympathetic activation and release of excess of catecholamines and alteration in the anti-inflammatory cholinergic parasympathetic nervous system, events that are likely to produce autonomic dysregulation and excessive serotonin inhibition that could trigger death. Hence, it is expected that plasma catecholamines, serotonin and acetylcholine levels (and also in the peripheral leukocytes, macrophages and T cells) will be abnormal in those who are at risk of death due to intensive insulin therapy. An increase in the circulating catecholamines and decrease in acetylcholine and serotonin is expected in these patients. Peripheral leukocytes, macrophages and T cells can be used for semiquantitation of catecholamines, acetylcholine and serotonin in these patients since they contain the complete intracellular machinery for the generation, release and inactivation of these molecules. Thus measurement of catecholamines, acetylcholine and serotonin in the plasma and peripheral leukocytes could form a simple and reliable method to predict those patients who are at high risk of

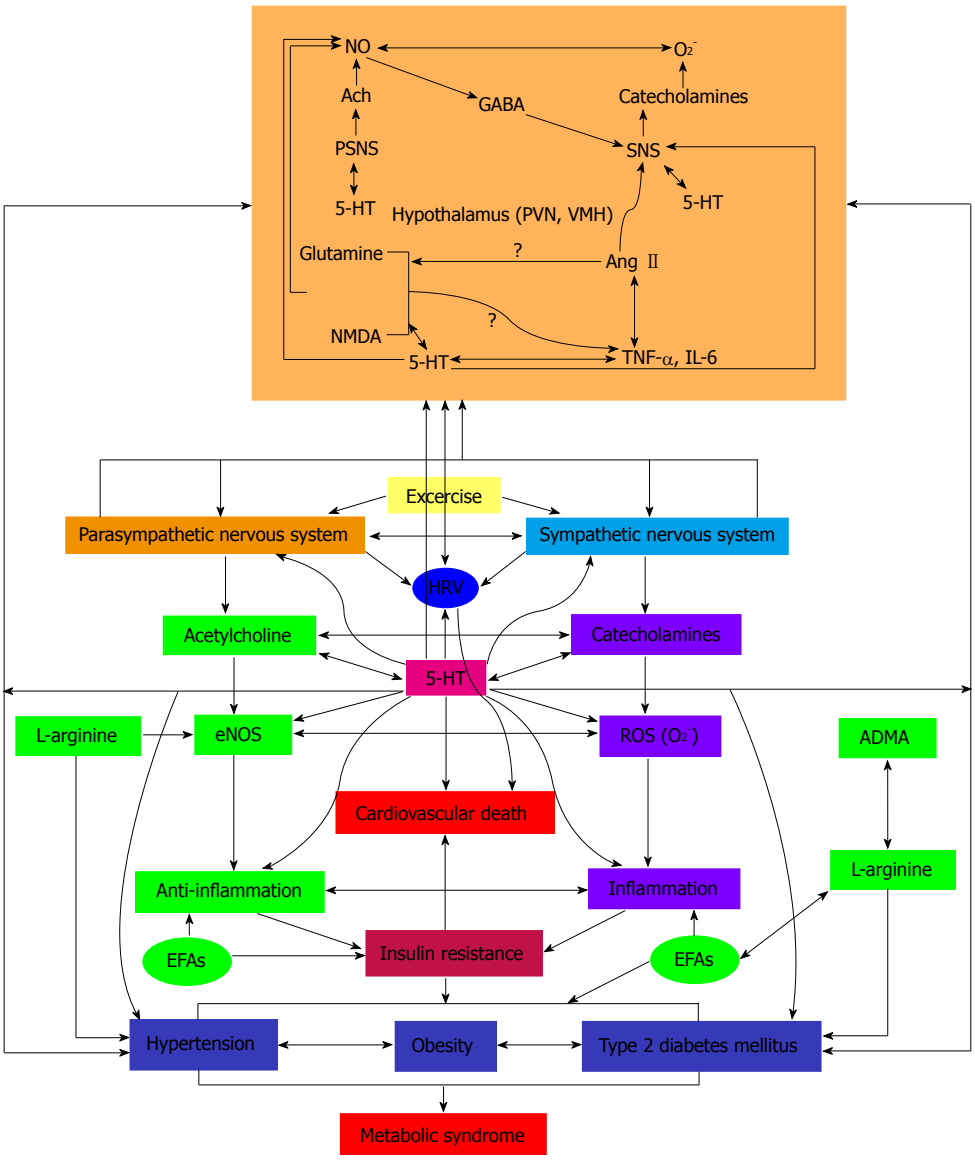

Figure 1 Scheme showing the interaction(s) among hypothalamic monoamines, autonomic nervous system, heart rate variability, inflammation, nitric oxide, essential fatty acids, dietary factors and type 2 diabetes mellitus and sudden cardiovascular death. Fasting, hypoglycemia and streptozotocin-induced diabetes alter brain tryptophan and serotonin metabolism. Serum norepinephrine and epinephrine increased following injection of insulin in both the ventromedial and lateral hypothalamus. In diabetes, an increase in norepinephrine level in the anterior and the medial-basal hypothalamus and a concomitant rise in dopamine content in the hypothalamus was reported. Serotonin levels were low in the hypothalamus in prediabetic animals. The decrease in serotonin level and its metabolites in the hypothalamus gradually decreased with the duration of diabetes. Brain insulin receptors and brain monoamines and acetylcholine interact with each other. In diabetics, the responses of the monoamines in the hypothalamus are inadequate to plasma and brain insulin levels. The serotonin content was higher in the hypothalamus while in pons medulla oblongata, serotonin and dopamine and the metabolites 5-hydroxy-3-indole acetic acid and DOPAC were significantly reduced whereas norepinephrine was markedly increased with a reduction in serotonin content in the hippocampus. In diabetes, the distal projections of serotonin were normal accompanied by hyperinnervation of the hypothalamus but the shorter collaterals were lost in the pons medulla oblongata due to lack/deficiency of insulin, changes that could result in an inappropriate response of hypothalamic monoamines to insulin. Lower Heart rate variability (HRV) occurs in young adults with metabolic syndrome much before the development of type 2 diabetes mellitus that suggests lower vagal activity and an increase in sympathetic predominance in type 2 diabetes and the critically ill that increases the cardiovascular risk. Hence, intensive insulin therapy may lead to alterations in the brain and peripheral monoamines, serotonin and acetylcholine levels that could cause sporadic autonomic dysregulation and excessive serotonin inhibition that may precipitate pronounced bradycardia and apnea and death. EFAs: essential fatty acids; eNO: endothelial nitric oxide; ADMA: asymmetrical dimethylarginine.

cardiovascular death due to intensive insulin therapy for the control of hyperglycemia.

CONCLUSION

The observation that tight glycemia led to significantly more deaths in the intensive therapy arm of the studies[8-12] in contrast to the expectation is rather puzzling. In both alloxan and streptozotocin-induced diabetic ani mals, an increase in norepinephrine and dopamine levels and a decrease in serotonin in the hypothalamus were reported[20,21]. In contrast, serotonin levels fell in all the parts of hypothalamus in prediabetic animals[21]. The contents of serotonin metabolites, 5-hydroxyindoleacetic

acid and homovanillic acid, in the whole brain gradually decreased with the duration of diabetes[21]. Intracerebro ventricularly (icv) administered insulin in doses induced minimal hypoglycemia, decreased brain dopamine and norepinephrine levels and produced an insignificant increase in serotonin concentration; and induced marked increase in rat brain acetylcholine levels that was accentuated in hyperglycaemic animals[22]. Thus, insulin appears to restore the altered brain monoamines to normal. The very specific changes in the monoaminergic innervations of the central nervous system seen both in obesity and type 2 diabetes mellitus[23] could be attributed to deficiency of insulin. It is likely that similar changes in monoamines could occur in patients with stress hyperglycemia since these subjects also have insulin resistance.

Hypoglycemic attacks that occur due to intensive insulin therapy, may, in turn, trigger sympathetic activation and release of excess of catecholamines and suppress anti-inflammatory cholinergic parasympathetic nervous system, events that are likely to produce autonomic dysregulation and excessive serotonin inhibition leading to death. Hence, it is expected that plasma catecholamines, serotonin and acetylcholine levels (and also in the peripheral leukocytes and T cells) will be abnormal in those who are at risk of death due to intensive insulin therapy. If this proposal is correct, methods designed to elevate serotonin levels, block α_2-adrenoreceptors (since blocking these receptor inhibits inflammation injury due to catecholamines[42]), stimulation of the vagus nerve[43] and the nicotinic acetylcholine receptor $\alpha 7$ subunit[44] are expected to be of significant benefit in the prevention of cardiovascular death in these patients.

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