CHAPTER V
DISCUSSION

In the present study, STZ-induced diabetic rats developed hyperglycemia, hypercholesterolemia, hypertriglyceridemia, hypertension, increased leukocyte-endothelial cells interaction, and impaired endothelium-dependent vasodilation. Interestingly, these abnormalities and impaired endothelium dependent vasodilation were prevented by long-term vitamin C supplementation.

PLASMA VITAMIN C LEVEL AND DIABETES MELLITUS

In agreement with other studies (Som S et al., 1994; Yew MS et al., 1983), our results demonstrate the significant decrease in plasma vitamin C in STZ-diabetic rats. The metabolism of vitamin C (ascorbic acid) is abnormal in diabetes. Especially, vitamin C concentration was reported to decrease in both plasma and tissues (Som S et al., 1994; Yew MS et al., 1983; Chen MS et al., 1983). Although, the decrease plasma ascorbic acid level has been well documented in both diabetic human and animal models, the mechanisms responsible for this has not yet been fully reported.

Ascorbic acid is transported across cellular membranes by two pathways. First, ascorbic acid itself is transported by a sodium-dependent saturable transporter. Second, ascorbic acid outside cells can be oxidized to dehydroascorbic acid (DHA), and then DHA is transported with the molecule of glucose by sharing the same protein transporter, especially GLUT 1 (NG LL et al., 1998). Once within cells, DHA is immediately reduced to ascorbic acid by both chemical and protein mediated processes

(Banhegyi G et al., 1997). Stankoval et al. (1984) have demonstrated that the uptake of radiolabelled DHA in leukocytes of diabetic patients was reduced. And the lower V_{max} of DHA uptake was also demonstrated. Moreover, in vitro study the chronic exposure of cells to high glucose level could lead to a reduction of V_{max} of DHA uptake. However, this effect was not due to the increased osmolarity (Ngkeekwong FC et al., 1997). The reduced uptake of DHA would further result to the reduction of the regeneration of ascorbic acid within the cell. Therefore, DHA, the oxidized from of ascorbic acid would be loss from the body via its hydrolysis in aqueous solution (Ng LL et al., 1998). Although, the uptake of ascorbic acid is similar bettween diabetic and non-diabetic subjects (Ng et al., 1998). However, deficiency of DHA-ascorbic acid recycle process could be conducted to the mechanism, for the reduction of plasma vitamin C in diabetes as summaried in Figure 31. (Ng LL et al., 1998). Therefore, the results showed in Table 3 have confirmed this hyperglycemic effect on the decrement of plasma level of Vitamin C.

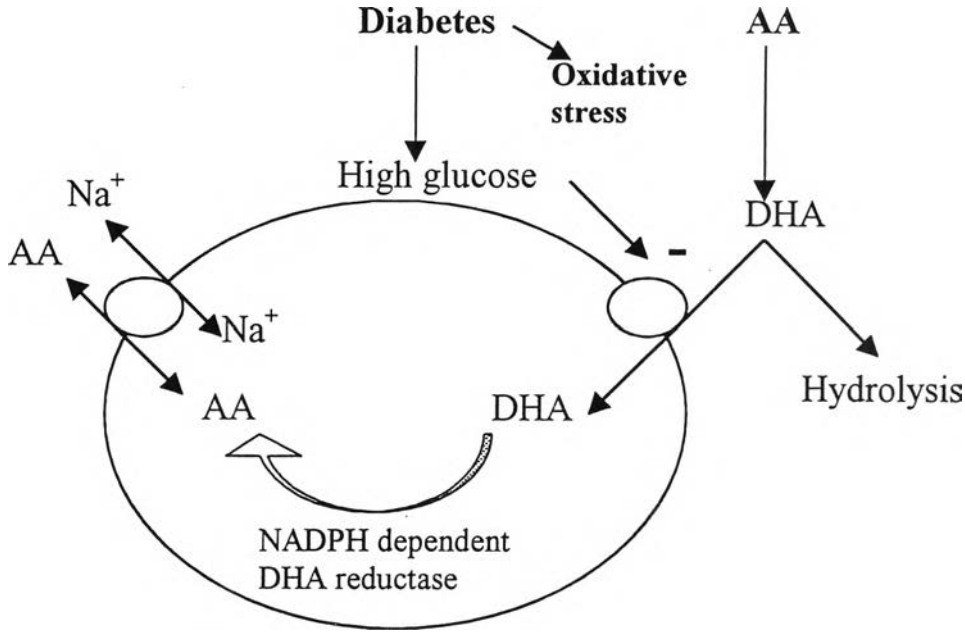

Figure 31. Scheme illustrating the major mechanism for vitamin C uptake and recycling involving DHA. DHA is generated from ascorbic acid (AA) in extracellular fluid due to oxidative stress, and in order to be regenerated by the intracellular enzyme DHA reductase. The DHA is then reduced to AA by cytoplasmic NADPH dependent DHA reductase so that the major intracelllular form of vitamin C is AA. Impairment of DHA uptake in diabetic cells especially in the presence of poor glycemic control leads to hydrolysis and irrecoverable loss of DHA, eventually resulting in depletion of AA stores (Diabetologia 1998;41:435-442)

1. EFFECTS OF LONG-TERM VITAMIN C SUPPLEMENTATION ON STREPTOZOTOCIN-INDUCED DIABETIC RATS : METABOLIC CHANGES

1.1 EFFECT ON HYPERGLYCEMIA

Up to now, hyperglycemia is very well documented as a cause of higher plasma free radical production (Ceriello., 1997), and the decrease in antioxidative defense mechanisms (Giugliano D et al., 1996). In the present study, after streptozotocin injection, hyperglycemia was developed. Moreover, plasma vitamin C reduction was also demonstrated in all monitored time points of STZ-diabetic rats. This study result was agree with the result reported by Cerielleo A. et al., (1998), which examined the antioxidant status in hyperglycemic state, by using an oral glucose tolerance test. They found that plasma concentrations of protein-bound sulhydryl groups, vitamin C, and uric acid decrease significantly.

They also suggested that the increased oxygen free radical production associated with a reduction in plasma antioxidants, particularly vitamin C and glutathione (GSH). In addition, free radicals and active oxygen species attack lipids, proteins, sugars, cleavage, cross-linking, and modification which eventually cause plasma membrane damage (Cross EC et al., 1987).

Interrestingly, the present study also demonstrated that the supplementation of vitamin C was significantly reduced hyperglycemic state (the results shown in Table 2). This unpredicted result was suggested by Paolisso G et al., (1994) that the beneficial effect of an acute rise in plasma

vitamin C was to increase insulin action and decrease plasma GSSG/GSH ratio.

They further suggested that increase in plasma vitamin C level with a simultaneous reduction in plasma GSSG/GSH ratio could enhanced in glucose transport. Therefore, their findings could give as an explanation to our results. Such that a significant increasing in plasma vitamin C in 36 wks STZ-vit C rats could cause the reduction of blood glucose. Although, in our study, streptozotocin was used to induced diabetic by damaging the β-cell, leading to deficient insulin secretion but several studies have shown that the intravenous injection of streptozotocin with 55 mg/kg BW. do not damage the whole β-cells. Thus small amount of plasma insulin can be detected in streptozotocin diabetic rats. As the model represents for hypoinsulinemic state. According to Paotisso G and Colleague, (1994) and our studies, the possibility mechanisms of vitamin C were that vitamin C may have the indirect effect to reduce blood glucose by scavenging free radicals, simultaneous increase in plasma GSSG/GSH ratio and leading to increasing insulin action, and then further decrease plasma glucose level.

1.2 EFFECTS ON PLASMA CHOLESTEROL AND TRIGLYCERIDE

The elevation of plasma cholesterol and triglyceride in STZ-diabetic rats reported by Dai S and Mc Neill (1995) were significantly confirmed in our study. Several observations have revealed that a deficient removal of circulating triglyceride and a decrease in the adipose tissue lipoprotein lipase play an important role in the pathogenesis of diabetic hyperlipidemia in man (Nikkila EA, 1974; Brunzell JD et al., 1979). In addition, decreased vitamin

C levels in plasma and tissues and common in diabetic patients and in animals with experimentally induced diabetes (Som S et al., 1981; Pecoraro RE et al., 1989; Dennis KY, 1989). Studied in guinea pigs fed a low vitamin C diet exhibited hyperlipidemia, whereas, those fed high vitamin C diet did not show abnormal plasma lipid level (Sharma P et al., 1990). Our present study, also showed that STZ-diabetic rats developed hyperlipidemia together with decreasing plasma vitamin C.

The mechanism of vitamin C in lipid metabolism and how decreased vitamin C causes hyperlipidemia are not clear. However, study in guinea pigs with chronic vitmin C deficiency had shown the slow down of transformation of cholesterol to bile acid, a principal catabolic product of cholesterol (Sharma P et al., 1990; Ginter E et al., 1978). Moreover, Ginter E (1978) has also revealed the benefit effect of vitamin C, or ascorbic acid, to restore the increased cholesterol level by increasing rate of conversion of cholesterol to bile acid. Moreover, dyslipidemia seen in many diabetic patients including high triglyceride, low HDL are associated with low lipoprotein-lipase (LPL) activity. Increased LPL activity can improved dyslipidemia in insulin deficient animals. LPL is rate limiting enzyme for removal triglyceride from the circulation and critical for the generation of HDL. Therefore, defects in LPL activity could exacerbate dyslipidemia in diabetes. It implied that the role of vitamin C supplementation on hyperlipidemia in diabetes may be involved the regulation lipoprotein lipase activity and transformation of cholesterol to bile acid.

2. EFFECTS OF LONG-TERM VITAMIN C SUPPLEMENT ON STREPTOZOTOCIN-INDUCED DIABETIC RATS

: HEMODYNAMIC CHANGES

2.1 EFFECTS ON MEAN ARTERIAL PRESSURE (MAP)

Vascular endothelium has a key role in maintaing homeostasis of the vasculature through the synthesis of vasoactive substances that modulate vascular tone (Moncada S et al., 1991; Garg UC et al., 1989). Endothelial dysfunction has been suggested to be an early event in diabetic microangiopathy (Porta M et al., 1987). Previous studies had suggested that there were the imbalances between the production/release/action of prostacyclin and nitric oxide as well as the elevated synthesis of angiotensin II and endothelin in diabetes (Ward KK et al., 1989; Maxfield EK et al., 1994; Cameron NE et al., 1994). These imbalances lead to the reduction of local vasodilation and more vasoconstriction been promoted. Therefore, in our results, such that, the significant elevation of mean arterial pressure could be accessed in STZ rats since 8 weeks after the STZ injection. However, our results also demonstrated that the supplementation of vitamin C could significantly reduced MAP as compared with STZ rats. At this point, it could be implied that the diabetic induced-endothelial dysfunction might be attenuated by the supplementation of vitamin C. In 1988, Salonen et al. observed a positive association between plasma vitamin C and serum 6-ketoprostaglandin-$F_{1\alpha}$. Interestingly, they have suggested that vitamin C could enhance the synthesis of prostacyclin since vitamin C could scavenging free radicals and peroxides, especially, the superoxide anion (Salonen JT et al., 1987) In other words, the possible mechanism of vitamin

C supplementation to prevent the elevation of MAP in STZ-diabetic rats due to its ability to prevent the endothelium to become dysfunction in particular through with scavenging oxygen-derived free radicals.

5. EFFECT OF VITAMIN C ON ARTERIOLAR FLOW RATE

In agreement with our results, Cotter MA and colleague demonstrated that the reduction in nutritive endonurial blood flow were observed in STZ-diabetic rats (Cotter MA et al., 1995). Interestingly, our results have demonstrated that the reduced cerebral arteriolar flow rate could be prevented by vitamin C supplementation. The mechanisms responsed for this reduction of cerebral arteriolar flow rate in diabetes has not yet been clarified. However, as we have discussed previously that there was the imbalance between endothelium-derived vasodilators (nitric oxide, prostacycline) and endothelium-derived vasoconstrictors (angiotensin II, endothelin) reported well in diabetic rats (Ward KK et al., 1989; Maxfield EK et al., 1994; Cameron NE et al., 1994). And this imbalance is believed to result from the increase in oxygen-derived free radicals. Moreover,there is an evidence showed that the increase vasoconstrictor could lead to reduce local organ blood flow (Cotter MA et al., 1995). Salonen et al., have suggested that vitamin C enhanced the synthesis of prostacyclin by scavenging free radical (Salonen JT et al., 1988).

Besides, Helmke BP et al. (1997) have the other point of view that the leukocyte adhesion in postcapillary venules may restrict organ blood flow by inducing vasoconstriction in the neighboring arterioles. Moreover, organ blood flow resistance may also be increased by hydrodynamic interactions between leukocytes and erythrocytes in the microcirculation

(Wikstrom TM et al., 1987). In our study, increasing of leukocytes adhere to the vascular endothelium were found in postcapillary venules in streptozotocin-diabeteic rats. Therefore, the increase in leukocyte adhesion and the reduction of arteriolar flow rate might be correlated through these all possible interaction. However, both of them share the same basis initiated primarily by the oxygen free-radical inducing endothelial dysfunction. Such that is the reason why the vitamin C supplementation could be used as an antioxidant in order to prevent both abnormalities.

Thus, the possible mechanism for vitamin C prevent the reduction of arteriolar flow rate may be results by: 1) increasing vasodilator production 2). reducing the increased vasoconstriction by prevention the increased leukocyte adhesion to vascular endothelium. Therefore, from the present results let us suggest that vitamin C supplementation prevented a reduction of arteriolar flow rate may mediate through its antioxidant effect.

II . EFFECTS OF VITAMIN C ON LEUKOCYTE-ENDOTHELIAL INTERACTION

VITAMIN C AND LEUKOCYTE ADHESION

The present results suggest a preventive effect of long-term vitamin C supplementation on leukocyte adhesion to endothelial cells in diabetic rats through its antioxidant capacity. The suggestion is supported by the following observations. First, the number of leukocyte adhesions were significantly increased in STZ-diabetic rats. Second, long-term vitamin C supplementation reduced the increasing of leukocyte adhesion in STZ-vit C rats.

In vitro studies, they have shown that monocytes isolated from diabetic patients were more adhesive to cultured human endothelial cells than those from healthy control subjects and that this leukocyte-endothelial cell adhesion was CD11-CD18 dependent (Dosquet C et al., 1999). From in vivo studies, leukocyte accumulation in the endothelium was increased in rabbits with alloxan-induced diabetes. Vlassara M, has demonstrated while capillary occlusions by monocytes and granulocytes it preceeded the destruction of capillary bed in diabetic retinopathy of rats (Hadcocks et al., 1991). Furthermore, Freedman SF and Hatchell DL demonstrated that enhanced superoxide radical production by stimulating leukocytes and increasing leukocyte adhering in the endothelium.(Schroder S et al., 1991).

The endothelial cell injury induced by activated leukocytes is primarily caused by the release of various leukocyte-produced factors, such as active oxygen species, cytokines and metabolites of arachidonic acid. Among these substances, it has been accepted that active oxygen species have the most injurious effect on endothelial cells (Weiss SJ et al., 1981). The mechanism of diabetes leads to leukocyte-adhesion to endothelium are only partially known. Kim JA and co-workers (1994) have demonstrated that hyperglycemia is thought to accelerate the increasing number of adhering monocytes to endothelial cell, by incubation of endothelial cells in vitro to high levels of glucose. In the other points, in experimental animals, a diet-induced hypercholesterolemia was shown to enhance adhesion of monocytes to the vascular endothelium (Joris I et al., 1983). Moreover, Hoogerbrugge N. et al (1996) studied in diabetic patients with a hypertriglyceridemia, the percentage of monocytes that adhere to endothelial cells in vitro was significantly increased compared with normolipidemic diabetic patients. Our results are greement with those previous studies that

the STZ-diabetic rats were developed hyperglycemia, hyperlipidemia and concomitance with significantly increased leukocyte adhesion to endothelial cells.

Recent studies shown that in diabetes, hyperglycemic itself, or the products formed by the interaction of glucose with cellular components, such as advanced glycosylated products (AGP_S) (Ruderman NB et al., 1992) may affected the expression of surface adhesion, and lead to leukocyte adhesion. In endothelial cells culture, the upregulation of adhesive molecule on cultured endothelial cells has been demonstrated after the exposure to AGP_s. Specifically, the enhanced expression of ICAM-I, VCAM-1 and E-selectin were observed on endothelial cells grow on AGE-modified matrix protein (fibronectin) (Morigi M et al., 1998).

Moreover, Weiss SJ. et al.,(1998) demonstrated that there is an excess generation of superoxide anion (O_2^-) within hypercholesterolemic vessels. Superoxide anion has been implicated in the oxidation of LDL (OxLDL) (Steinbrecher UP, 1988). The previous study has demonstrated the potential of OxLDL to induce adhesion of leukocytes to endothelial cells. The action of superoxide radicals and other reactive oxygen species can be counteracted not only by superoxide dismutase, but also by the water-soluble antioxidant vitamin C (Lehr HA et al., 1994; Frei B, 1999). Vitamin C has been demonstrated to interfere with the process of lipid peroxidation by scavenging superoxide anion (Heitzer T et al., 1996).

Therefore, from our result, we suggested that the generation of oxygen-derived free radicals from both hyperglycemia and hyperlipidemia are the major contributors to induce increasing leukocyte adhesion in STZ-

diabetic rats. And vitamin C can prevent leukocyte adhesion by its antioxidant effect.

III. EFFECTS OF VITAMIN C ON RESPONSES OF CEREBRAL ARTERIOLES TO ENDOTHELIUM-DEPENDENT AND-INDEPENDENT VASODILATORS

ANTIOXIDANT EFFECTS OF VITAMIN C ON ENDOTHELIAL FUNCTION

The important new finding has recently demonstrated that the responses of cerebral arterioles in STZ-diabetic rats to acetylcholine(Ach) and adenosine-5′ diphosphate(ADP) were significantly decreased compared with non-diabetic control rats. Vasodilation response of rat cerebral arterioles to Ach and ADP depend on endothelial nitric oxide (NO) production. Whereas, the response to nitroglycerine (NTG), the exogenous NO donor, which can promote vasodilation directly through the activating vascular smooth muscle soluble guanylate cyclase, were not altered by diabetes. Therefore, it is clearly demonstrated that the impairment is occurred specifically on the endothelial cell. The new finding of our study is that long-term supplementation of vitamin C can significantly prevent this impairment of endothelium. The preventive effect of vitamin C probably mediated by the ability of it to scavenge oxygen-derived free radicals, especially superoxide anion.

The endothelium plays an important role in maintaining vascular tone and function in part by the synthesis and release of vasoactive substances such as nitric oxide (NO) (Furchott RF et al 1989; Vanhoutte PM et al 1989). One possible mechanism that contributes to endothelial dysfunction

in diabetes mellitus is the decrease in endothelium-derived nitric oxide. Perhaps through the accumulation of oxygen-derived free radicals.

The development of endothelial cell dysfunction is characterized by an impairment in vasodilation and increased adhesiveness of the endothelial cell lining. Several lines of evidence indicate that endothelial cell dysfunction is associated with alterations in the cell redox state. More recent experimental and clinical data strongly demonstrate evidence for increase oxidative stress being responsible for endothelial dysfunction in diabetes mellitus (Kimi JA et al., 1994, Weiss SJ et al., 1981). Oxidative stress induced by hyperglycemia is implicated as a source of altered endothelium vasodilation in diabetes. Tesfamariam and Cohen (1992) have shown that elevated glucose concentrations impaired Ach stimulated endothelium induced vascular smooth muscle relaxation in vitro and that this impairment can be reversed by antioxidants, which include superoxide dismultase (SOD), catalase.

In addition, the recent evidence has mentioned that the particular elevation of glucose could play the major role on oxidative modification of LDL by a superoxide-dependent pathway. Moreover, it has been demonstrated that in IDDM patients who have poorly control of blood glucose will have the increased LDL oxidation (OxLDL). Especially, this increased OxLDL was in relation to the reduced antioxidant defenses. Therefore, OxLDL may cause endothelial damage and reduce nitric oxide synthesis (Meister A, 1994).

Many findings demonstrated that the enhanced production of oxygen-derived free radical promote the impairment of endothelium-dependent vasodilation by several mechanisms, including inactivation of nitric oxide decreasing production of nitric oxide, reaction with nitric oxide, to from proxynitrite, a reactive radical species that can also be converted to the highly reactive hydroxyl radical (Tesfamariam B 1994, Tesfamariam B and Cohen RA, 1992).

From the present result, long-term vitamin C supplementation can prevent impairment of endothelial-dependent vasodilation in STZ-vit C rats. Long-term supplementation of the antioxidant, vitamin C significantly prevented impairment of endothelium-dependent vasodilation to Ach and ADP. This preventive effect of vitamin C probably mediated by the ability of it to scavenge excess superoxide anions, and prevent nitric oxide inactivation. Nishikimi N (1975) studied the effect of vitamin C in vitro model, he demonstrated that vitamin C can protect the oxidative stress by scavenging excess superoxide anion.

In our study, the chronic STZ-diabetic rat also developed hypercholesterolemia and hypertriglyceridemia. Insulin dependent diabetic patients who are poorly controlled have elevated level of total triglyceride, cholesterol, LDL-cholesterol, and VLDL-cholesterol (Brunzell JD et al., 1979). High or borderline elevation of total cholesterol is evident in a high percentage of diabetic patient and experimental diabetes (Brunzell JD et al., 1979, Nikkila EA 1974). These may cause further rising of LDL-cholesterol The susceptibity of LDL to oxidative modifice has also been reported to be increase in diabetes (Nikkila EA 1974). Oxidized LDL are atherogenic by many mechanisms, such as direct chemotactive activity for monocytes, smooth muscle cells, inhibition of the production and biologic activity of

endothelium-derived nitric oxide, and cytotoxicity (Som S et al., 1981) . There is evidence from in vitro studies that physiological concentrations of vitamin C strongly inhibit LDL oxidation (Som S et al., 1981; Pecoraro RE et al., 1989). Moreover, vitamin C prevents oxidative modification of LDL primarily by scavenging free radicals and other reactive species in the aqueous phase (Ginter E et al., 1978) Thus, the direct trapping of vitamin C might be able to prevent both interaction between oxygen-free radicals and LDL and also the formation of oxidized LDL.

Therefore, it is hypothesized that the possible mechanisms by which vitamin C might prevent the impairment of endothelial-derived vasodilation, included 1) scavenging of oxygen-derived free radical especially superoxide radical, 2) decreasing toxic interactions of endothelial cells and LDL by preventive oxidation of LDL.

IV. THE EFFECT OF VITAMIN C SUPPLEMENTATION ON ULTRASTRUCTURAL CHANGES OF CEREBRAL MICROVESSELS

One of the major histopathological features of diabetes is a widespreard thickening of vascular basement membranes. Most evidence indicates that diabetic basement membranes contain smaller-amounts of proteoglycans than their normal counter parts and perhaps to compensate for this defect, larger amounts of type IV collagen and laminin (Strnberg M et al., 1985; Spiro RG, 1973). In streptozotocin-induced diabetes rats, glomerular basement membrane type IV collagen appears to be concentrated in the damina rara interna. (Bendayan M, 1985). There are also increases in serum concentrations of type IV collagen and laminin (Cohen MP et al.,

1982; Reddi AS, 1985) and a reduction in the rate of glomerular basement membrane turnover in diabetes. One factor that caused increase basement membrane thickening is AGEs. AGEs on matrix proteins can also crosslink adjacent matrix components. Cross linking by AGEs has detrimental effects on other important properties of matrix proteins, leading to disorder in the interaction between type IV collagen, laminin, heparan sulphate, proteogly can, and entactin (Tarsio JF et al., 1987).

Once, AGEs are closely related to product of oxidative protein modification because of their potency of antoxidation and also produces free radicals (Baynes JW, 1991). Moreover, Vitamin C supplementation could prevent the thickness of vascular basement membrane of STZ-Vit C rats for all monitored time point. It can imply that the effect of vitamin C to prevent these abnormalities might be through its antioxidant effect.

Proposed mechanism for the antioxidant effects of vitamin C on Endothelial dysfunction in Diabetes Mellitus of the present study

Based on intravital microscopic and electron microscopic studies on cerebral microvasculature in non-diabetic, STZ-diabetic rats with and without vitamin C supplementation. The aim of present study was examined the antioxidant effect of vitamin C on endothelial dysfunction in STZ-diabetic rats. In present results, the alterations of both functional and morphological of cerebral microvasculature, including impaired endothelial-dependent vasodilation and the increased leukocyte adhesion to endothelial cells are dominant characters for indicating endothelial dysfunction. The consequences followed the endothelial dysfunction which were demonstrated in the present study are the elevated mean arterial pressure, the

reduced arteriolar flow rate and the increased thickness of cerebral capillary and arteriolar basement membrane.

Interestingly, vitamin C can significantly prevent the endothelial dysfunction in STZ-diabetic rats. It is well documented that vitamin C is an aqueous phase antioxidant. Among its antioxidant properties, vitamin C has been shown to be an efficient scavenger of reactive oxygen species, including superoxide radical. Therefore, it has been suggested that cerebral endothelial dysfunction in STZ-diabetic rats results from cerebral oxygen-derived free radical.

In agreement with other studies, the present results demonstrate that both hyperglycemia and dyslipidemia including hypercholesterolmia and hypertriglyceridemia are major contributors to produce oxygen-derived free radical. In additon, high elevation of total cholesterol is evident for increasing LDL-cholesterol . The oxidation of LDL to oxygen derived free radical (OxLDL) has been reported to be increase in diabetes. Both OxLDL and oxygen-derived free radical have potentially damaged endothelial cells, leading to inactivate endothelium derived nitric oxide, decrease prostacyclin synthesis, and increase production of vasoconstrictors such as angiotensin II, and endothelin.

We belived that the imbalance of endothelium-derived-vasodilators and-vasoconstrictors are caused of elevation in MAP and decreased arteriolar flow rate. All the above results, it suggests that one possible mechanism by which vitamin C could prevent endothelial dysfunction included: 1) scavenging of oxygen-derived free radical, especially superoxide radical, decreasing the toxicity of interactions between endothelial cells and LDL by preventing oxidation of LDL. Finally, hypoglycemic effect of vitamin C should be emphasized in the present

study. We suggest this hypoglycemic effect of vitamin C by preventing damaged plasma membrane from oxygen free radical, leading to increase glucose transport. In addition, increase plasma vitamin C after long-term vitamin C supplementation leads to the increase of both secretion and action for insulin

In conclusion, long-term-supplementation of the antioxidant, vitamin C, prevented endothelium-dependent vasodilation in STZ-diabetic rats. This finding can be suggested that oxygen-derived free radicals contribute to abnormal vascular function in diabetic mellitus Therefore, the efficiency of long-term vitamin C supplementation in preventing endothelial dysfunction, may be an important therapeutic implication used for the prevention of the risk of vascular disease especially, cerebrovascular disease, including stroke.

Proposed mechanisms of Vitamin C on Endothelial function in Diabetes mellitus

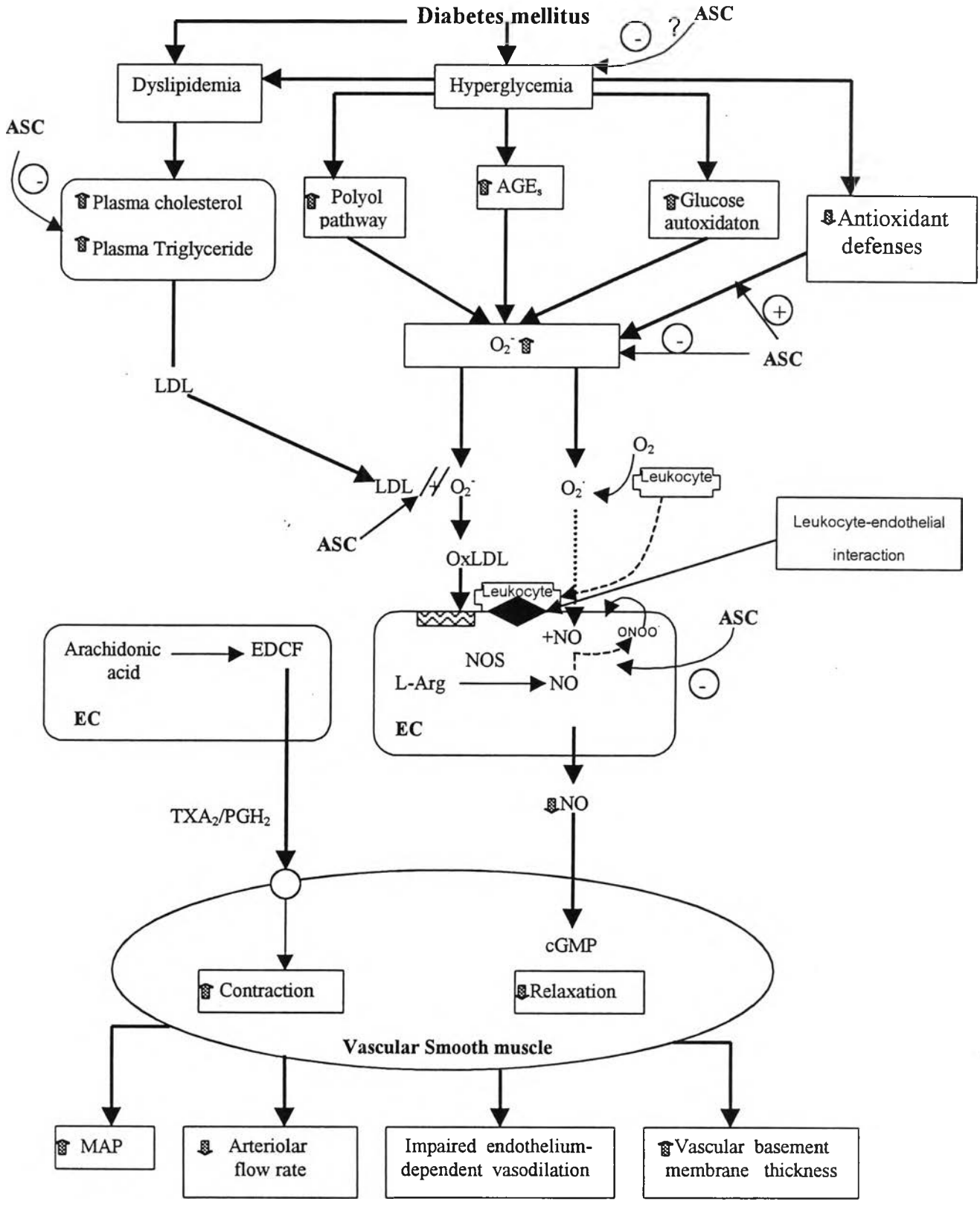

Figure 32. The proposed mechanism of vitamin C on endothelial cells in diabetes mellitus