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# Effect of tetrahydrocurcumin on insulin receptor status in type 2 diabetic rats: studies on insulin binding to erythrocytes

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Curcumin is the most active component of turmeric. It is believed that curcumin is a potent antioxidant and anti-inflammatory agent. Tetrahydrocurcumin (THC) is one of the major metabolites of curcumin, and exhibits many of the same physiological and pharmacological activities as curcumin and, in some systems, may exert greater antioxidant activity than curcumin. Using circulating erythrocytes as the cellular mode, the insulin-binding effect of THC and curcumin was investigated. Streptozotocin (STZ)–nicotinamide-induced male Wistar rats were used as the experimental models. THC (80 mg/kg body weight) was administered orally for 45 days. The effect of THC on blood glucose, plasma insulin and insulin binding to its receptor on the cell membrane of erythrocytes were studied. Mean specific binding of insulin was significantly lowered in diabetic rats with a decrease in plasma insulin. This was due to a significant decrease in mean insulin receptors. Erythrocytes from diabetic rats showed a decreased ability for insulin–receptor binding when compared with THC-treated diabetic rats. Scatchard analysis demonstrated that the decrease in insulin binding was accounted for by a decrease in insulin receptor sites per cell, with erythrocytes of diabetic rats having less insulin receptor sites per cell than THC-treated rats. High affinity ( $K_{d1}$ ), low affinity ( $K_{d2}$ ) and kinetic analyses revealed an increase in the average receptor affinity of erythrocytes from THC-treated rats compared with those of diabetic rats. These results suggest that acute alteration of the insulin receptor on the membranes of erythrocytes occurred in diabetic rats. Treatment with THC significantly improved specific insulin binding to the receptors, with receptor numbers and affinity binding reaching near-normal levels. Our study suggests the mechanism by which THC increases the number of total cellular insulin binding sites resulting in a significant increase in plasma insulin. The effect of THC is more prominent than that of curcumin.

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## 1. Introduction

A number of recent studies have shown that human erythrocytes can be used as a cellular model for assessing the status of insulin receptors in diabetes (Pari *et al* 2004; Ashokkumar *et al* 2006). Since erythrocytes can be easily obtained in sufficient numbers from serial studies, they have been used as a model for insulin-binding studies (Pollet *et al* 1981; Grigorescu *et al* 1983; Susheela *et al* 1987). Human erythrocytes are readily accessible and are therefore often

used for studying insulin receptor regulation in states of altered insulin action (Dons *et al* 1981). The characteristics of insulin binding to mature erythrocytes are similar to those of other cells in terms of affinity, temperature dependence, optimum pH, specificity and negative cooperativity (Gambhir *et al* 1978). Under steady-state conditions, there appears to be a good correlation between the insulin receptors on erythrocytes and that in other tissues (Dons *et al* 1981). The affinity of erythrocyte insulin receptors does show variation, which parallels that in monocytes and

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Abbreviations used: B/F ratio; bound/free ratio; DMRT, Duncan multiple range test; ELISA, enzyme-linked immunosorbent assay; K, aver-

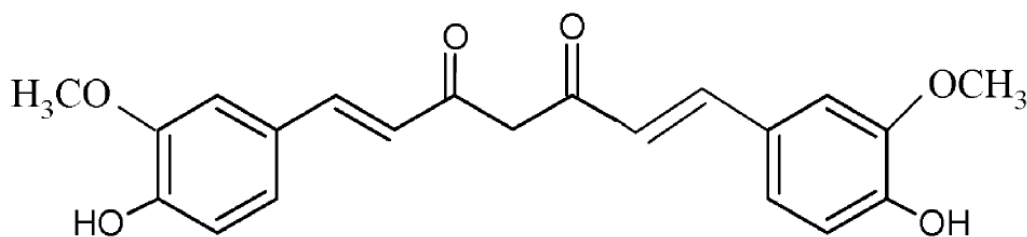
exhibits acute modulation *in vivo* in response to intravenous infusions of insulin or glucose (Insel *et al* 1980).

Erythrocytes should therefore be useful for reflecting changes in other tissues, in terms of changes in insulin receptor affinity and long-term changes in receptor concentration (Ward and Harrison 1986). Ligand–receptor binding studies are widely used for receptor characterization and high-throughput drug screening. Receptor binding is used to characterize receptors and evaluate potential pharmaceutical agents by assessing their ability to interfere with the specific binding of a radiolabelled ligand to its receptors. Using the radioreceptor assay of Gambhir *et al* (1978), we investigated the effect of tetrahydrocurcumin (THC) on  $^{125}\text{I}$ -insulin binding to the erythrocyte receptors of normal and experimental rats, and determined whether the plasma insulin concentration could affect the number and/or affinity of insulin receptor sites according to the down- and upregulation theory.

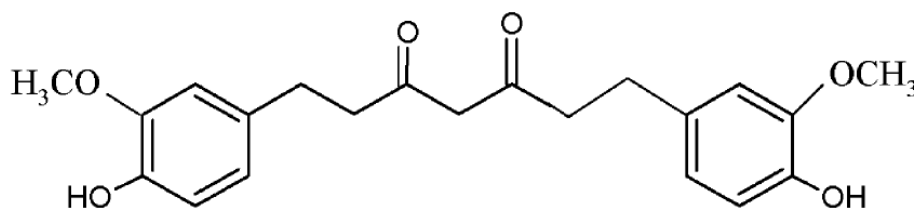
Curcumin (diferuloylmethane) is the substance that gives the yellow colour to turmeric, a spice that is extensively used in Indian cuisine as a component of curry powder. Curcumin is extracted from the roots of the *Curcuma longa* plant (turmeric). It is believed that curcumin is a potent antioxidant and anti-inflammatory agent. Practitioners of traditional Indian medicine believe that curcumin powder is beneficial against many diseases including biliary disorders, diabetes, hepatic disorders, rheumatism, sinusitis, cancer

(Aggarwal *et al* 2003) and Alzheimer disease (Garcia-Alloza *et al* 2007). In experimental studies, curcumin has been shown to reduce hyperlipidaemia (Babu and Srinivasan 1997), delay the development of cataract (Suryanarayana *et al* 2005), ameliorate renal lesions (Babu and Srinivasan 1998), and reduce the cross-linking of collagen (Sajithlal *et al* 1998) in a streptozotocin (STZ)-induced diabetic animal model. Curcumin has also been shown to lower blood glucose levels in type 2 diabetic KK-Ay mice (Nishiyama *et al* 2005) and STZ-treated rats (Mahesh *et al* 2005). Curcumin supplementation promotes wound healing in STZ-treated diabetic rats and in genetically diabetic mice (Sidhu *et al* 1999), and also attenuates the phenylephrine-induced increase in vascular reactivity of the aorta in STZ-treated diabetic rats.

Tetrahydrocurcumin (THC) is one of the major metabolites of curcumin. Structurally, THC and curcumin (figure 1) have identical  $\beta$ -diketone structures and phenolic groups, but differ in that THC lacks the double bonds of curcumin (Okada *et al* 2001). Recently, attention has been focused on THC, because this compound appears to exert a greater antioxidant activity in both *in vitro* and *in vivo* systems (Okada *et al* 2001; Pari and Murugan 2004). Sugiyama *et al* (1996) demonstrated that THC exhibited similar physiological and pharmacological properties as the active form of curcumin *in vivo*. Naito *et al* (2002) showed clear involvement of THC in biochemical and



Structure of curcumin



Structure of tetrahydrocurcumin

**Figure 1.** Chemical structures of curcumin and its major metabolite, tetrahydrocurcumin (THC). Curcumin and THC have similar  $\beta$ -diketone structures and phenolic groups.

molecular actions at the cellular level in ameliorating oxidative stress in cholesterol-fed rabbits. Some researchers also found that curcumin has a neuroprotective role in amyloid neurotoxicity and amyloid fibril formation in Alzheimer models and other neurodegenerative diseases (Lim and Lin-Shiau 2001). Furthermore, Okada *et al* (2001) have claimed that THC has more potent antioxidant activity than curcumin.

Novelli *et al* (2001) suggest that following STZ and nicotinamide administration, a partial loss of the  $\beta$ -cell mass occurs by necrosis and/or apoptosis, induced by the relatively specific cytotoxic effect of STZ, which is only partially counteracted by nicotinamide. The residual  $\beta$ -cells (about 60% of the original mass) are most likely those that escaped irreversible damage and maintained the differentiation of mature  $\beta$ -cells (Novelli *et al* 2001). Oral hypoglycaemic agents are used in non-insulin dependent diabetes mellitus to stimulate the pancreatic  $\beta$ -cells to secrete insulin and/or increase the sensitivity of peripheral insulin receptors to the action of endogenous insulin (Reusch 1998). The past few years have witnessed the introduction of a number of new oral agents for the treatment of type-2 diabetes, with the hope of achieving better glycaemic control.

Recently, in our laboratory, we found that THC improves plasma insulin, decreases glucose levels, scavenges free radicals and also has antioxidant activity in type 2 diabetic rats (Murugan and Pari 2006a,b, 2005). THC reverses the changes in the levels of carbohydrate moieties of glycoproteins (Pari and Murugan 2006), hyperlipidaemic effect and protects fatty acid composition (Murugan and Pari 2006c, 2007). However, the effects of THC on the insulin receptors in STZ-treated diabetic rats have not been studied, to the best of our knowledge. To better understand how THC produces an antihyperglycaemic effect, we analysed the effect of THC on the insulin-binding sites of erythrocytes in diabetic rats.

## 2. Materials and methods

### 2.1 Animals

Adult male albino Wistar rats (8 weeks), weighing 180–220 g and bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used. All animal experiments were approved by the ethics committee (vide No: 284, 2005), Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The animals were housed in polycarbonate cages in a room with a 12 h day–night cycle, temperature of  $24 \pm 2^\circ\text{C}$ , humidity of 45–64%. During the period of the experiment,

animals were fed with a balanced commercial diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

### 2.2 Drugs and chemicals

THC was gifted by Sabinsa Corporation, USA. Curcumin was purchased from Sigma Chemicals Company, St Louis, USA. All other chemicals and biochemical materials were of analytical grade.

### 2.3 Induction of diabetes

STZ was dissolved in citrate buffer 0.1 M (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Type 2 diabetes mellitus was induced in overnight-fasted rats by a single intraperitoneal injection of 65 mg/kg STZ, 15 min after the intraperitoneal administration of 110 mg/kg of nicotinamide (Masiello *et al* 1998). Hyperglycaemia was confirmed by elevated plasma glucose levels determined at 72 h. Animals with a blood glucose concentration of more than 250 mg/dl were used for the study.

### 2.4 Experimental design

In the experiment, a total of 24 rats (18 diabetic surviving rats, 6 normal rats) were used. The rats were divided into four groups of six each, after the administration of STZ to induce diabetes in 18 of 24 rats. The experimental period was 45 days. Group I comprised normal untreated rats, while group II comprised diabetic control rats. Rats belonging to groups III and IV were diabetic and were given THC and curcumin, respectively, daily for 45 days (80 mg/kg body weight in aqueous suspension) through an intragastric tube.

No detectable irritation or restlessness was observed after administration of any drug or vehicle. No noticeable adverse effect (i.e. respiratory distress, abnormal locomotion or catalepsy) was observed in any animal after drug administration. At the end of 45 days, all the rats were killed by decapitation after inducing anaesthesia (pentobarbitone sodium, 60 mg/kg). Blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose, and plasma was separated for assay of insulin.

### 2.5 Biochemical estimations

**2.5.1 Measurement of blood glucose and plasma insulin:** Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics [I] Pvt. Ltd, Baroda, India) (Lott and Turner 1975). Plasma insulin was assayed by enzyme-linked immunosorbent assay

(ELISA) using a Boehringer–Mannheim kit with an ES300 Boehringer analyser (Mannheim, Germany).

**2.5.2 Preparation of purified erythrocytes:** Erythrocytes were separated using a Percoll density gradient. Mononuclear leukocytes were separated from the erythrocytes by the use of Pasteur pipettes. The receptor assay for erythrocytes was performed according to a modification of the method of Gambhir *et al* (1978). The erythrocytes were washed thrice by centrifugation (4°C, 4500 rpm) in 10 ml of buffer G containing (in mmol/l) tris (hydroxymethyl) methylamine, 50; 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid, 50; MgCl<sub>2</sub>·6H<sub>2</sub>O, 10; CaCl<sub>2</sub>, 10; ethylenediaminetetraacetic acid, 2; dextrose, 10; NaCl, 50; KCl, 5; and 1% human serum albumin (pH 7.8) for 10 min. On each occasion, the supernatant was removed, the cells resuspended in buffer G and respun. After the final washing of the cells, the supernatant was removed and the cells were left in 4 ml of buffer G containing 1% human serum albumin. This suspension contained 4–6 × 10<sup>9</sup> cells/ml.

**2.5.3 Binding of <sup>125</sup>I to erythrocytes:** Erythrocytes (4.5 × 10<sup>9</sup> cells/ml) were incubated at 15°C with <sup>125</sup>I-insulin (40 pg in 25 μl) with or without varying amounts of unlabelled insulin (0–0.5 × 10<sup>5</sup> ng) in a total volume of 0.5 ml. After 2.5 h of incubation, duplicate samples were placed in pre-chilled microfuge tubes along with the buffer and dibutylphthalate. Cell-bound and free insulin were separated by centrifugation at 7000 g at 4°C for 10 min. The radioactivity in the cell pellet and supernatant were analysed in a gamma counter (ECIL, Hyderabad). The data were analysed by Scatchard analysis (Scatchard 1949). Receptor affinity and receptor numbers were derived for the physiological range of insulin, i.e. between 0.1 and 100 ng/ml. Specific insulin binding was calculated as the percentage of radioactive insulin bound by 4 × 10<sup>9</sup> cells/ml for erythrocytes. Non-specific binding is defined as the amount of radioactive insulin that remains bound in the presence of 10<sup>5</sup> ng/ml of unlabelled porcine insulin. All binding data were corrected for non-specific binding to represent specific cell binding for the purposes of comparison.

Competitive binding curves were obtained for each erythrocyte suspension. From these curves, the insulin receptor affinity and number of receptor sites were determined by the Scatchard analysis.

**2.5.4 Cell binding analysis:** The results of the binding studies are presented in three ways: (i) the percentage binding of <sup>125</sup>I-insulin as a function of the total insulin concentration (competitive curve) (De Pirro *et al* 1980), (ii) the bound–free insulin ratio plotted as a function of the bound insulin (Scatchard plot) and (iii) the average affinity profile (McElduff and Eastman 1981). The total binding capacity or concentration of the binding sites was derived from the point where the linear extrapolation of the curve

intercepted the horizontal axis and this was used to calculate the number of receptor sites per cell (Scatchard 1949).

## 2.6 Statistical analysis

All data were expressed as mean ± SD of number of experiments. Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 7.5 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by the Duncan multiple range test (DMRT) (Duncan 1957). A value of *P* < 0.05 was considered to indicate a significant difference between groups. The data on insulin-binding studies were analysed by competition curve, Scatchard plot and average affinity profiles. All values are expressed as mean ± SD.

## 3. Results

### 3.1 Effect of THC on blood glucose and plasma insulin

Figure 2 shows the level of blood glucose and plasma insulin of the various experimental groups. Diabetic control rats showed a significant increase in the blood glucose level with a significant decrease in the plasma insulin level. Oral administration of THC to diabetic rats significantly reversed the above biochemical changes. In our previous study (Pari and Murugan 2005) we reported that THC at a dose of 80 mg/kg body weight showed a better effect than doses of 20 and 40 mg/kg body weight; therefore, the dose of 80 mg/kg body weight was used in this study. The administration of THC and curcumin to normal rats had a significant effect on blood glucose and plasma insulin levels. THC administration was more effective than curcumin.

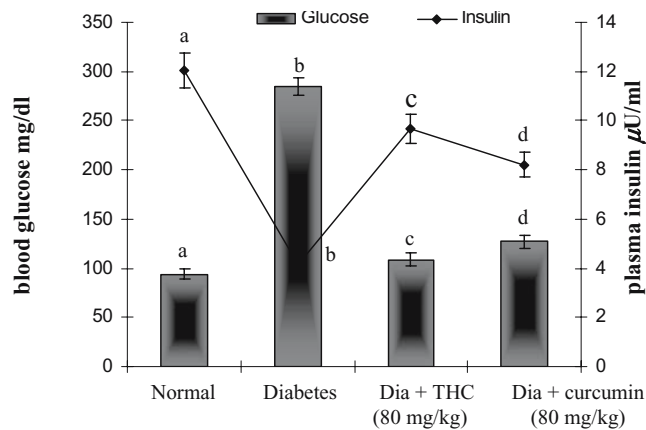
### 3.2 Effect of THC on diabetic changes in competitive binding curves on the binding of insulin to its receptors on erythrocytes

Figure 3 summarizes the ability of non-radioactive insulin to competitively inhibit the binding of <sup>125</sup>I-insulin to the insulin receptor on the cell membranes of erythrocytes in rats treated with STZ, THC and curcumin. The percentage of <sup>125</sup>I-insulin bound to the insulin receptor on the cell membrane of erythrocytes of diabetic rats was significantly lower than the percentage of <sup>125</sup>I-insulin bound to those of THC- and curcumin-treated diabetic rats at very low concentrations (0 and 1 ng/ml) of erythrocytes. Comparison of the competitive curves of the percentage of <sup>125</sup>I-insulin bound to the insulin receptor on erythrocytes of diabetic and THC-treated diabetic rats showed slopes that decreased

steadily at an insulin concentration of 10 ng/ml.

### 3.3 Effect of THC on diabetic changes in bound/free ratio

In this study, the bound/free (B/F) ratio of the labelled hormone was expressed as a function of the bound hormone, giving a Scatchard plot for erythrocytes (figure 4). Curvilinear



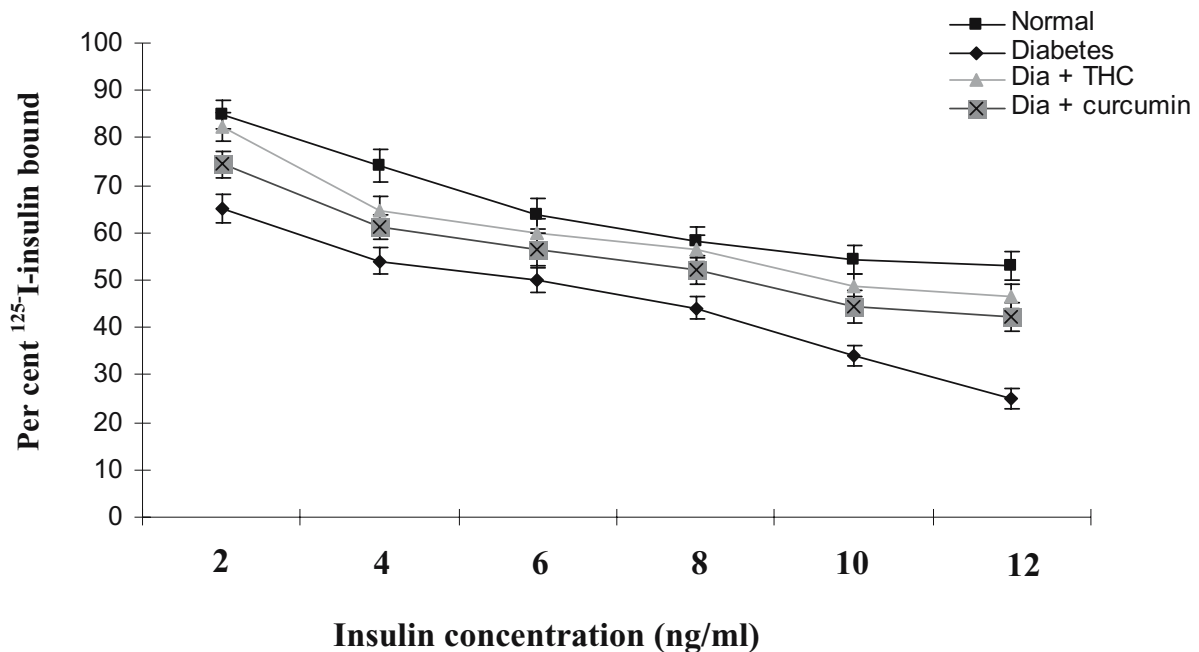
**Figure 2.** Effect of THC on the levels of blood glucose and plasma insulin in normal and experimental rats. Dia, diabetes; THC, tetrahydrocurcumin. Values are given as mean  $\pm$  SD for 6 rats in each group. Values that do not share a common superscript letter differ significantly at  $P < 0.05$  (DMRT).

plots were obtained for the diabetic control, THC-treated, curcumin-treated and normal rats. A greater B/F implies that there is more bound than free hormone. Comparison of the plots showed that the insulin receptors on the cell membranes of erythrocytes in THC-treated diabetic rats had maximum B/F values compared with those of diabetic rats.

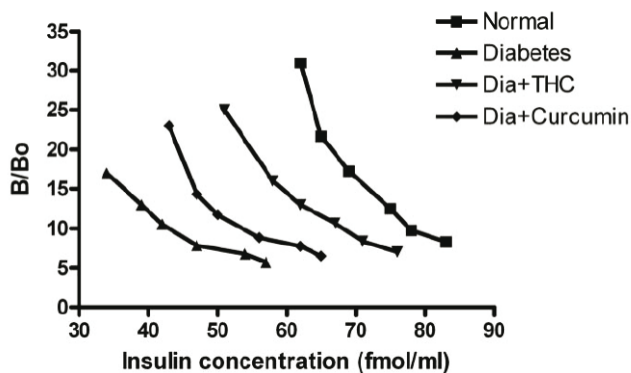
### 3.4 Effect of THC on diabetic changes in Scatchard analysis of affinity profile and receptor-binding sites in erythrocytes

To analyse the above changes in affinity more precisely, the  $K_d$ ,  $K_c$  and  $K_f$  were calculated. “Dissociation constant of high-binding sites” ( $K_{d1}$ ) was significantly decreased in diabetic rats and increased in diabetic rats treated with THC and curcumin (figure 5A). “Receptor numbers” ( $B_{m1}$ ) for  $K_{d1}$  were maximum in rats treated with THC and curcumin when compared with diabetic rats (figure 6). Further dissociation constant of low-binding sites ( $K_{d2}$ ) (figure 5B) showed significantly decreased affinity in the erythrocytes of diabetic rats and significantly increased affinity in rats treated with THC and curcumin.

Similarly, “receptor numbers” ( $B_{m2}$ ) (figure 6) also decreased in diabetic rats whereas those in whom THC was administered had a significantly increased  $B_{m2}$ . Analysis of the data shows that the erythrocytes of rats treated with THC and curcumin have an “empty site affinity” ( $K_c$ ), which begins to decrease with increasing occupancy of the



**Figure 3.** Competitive binding curves showing the effect of tetrahydrocurcumin (THC) and curcumin in diabetic rats on the binding of insulin to its receptor on erythrocytes. Dia, diabetes.



**Figure 4.** Scatchard plot showing the effect of tetrahydrocurcumin (THC) on the binding of insulin to its receptor on erythrocytes of normal and experimental rats. Bound/Free ratio is plotted as a function of the insulin-bound B/Bo. Dia, diabetes; THC, tetrahydrocurcumin

receptor sites by  $^{125}\text{I}$ -insulin. The average receptor affinity progressively decreased to the “filled site affinity” ( $K_f$ ) (figure 5C).

#### 4. Discussion

Diabetes mellitus is a metabolic disorder of heterogeneous aetiology (Olefsky and Kolterman 1981). Insulin binding to receptors is the first event signifying the action of insulin, and this first step represents a major control point for the effects of insulin *in vivo*. Insulin binding to receptors is not a fixed biological process but is subjected to modulation by alterations in either the receptor or affinity (De Pirro *et al* 1980). Insulin receptors have been demonstrated in cells of a large variety of tissues from different animal species. Many studies have shown that insulin binding is decreased in diabetes mellitus (Olefsky and Kolterman 1981; Kolterman *et al* 1981).

STZ-nicotinamide caused significant reduction in the number of receptors on erythrocytes and insulin target tissues. In the present investigation, treatment with THC showed significant antihyperglycaemic activity. Moreover, this indirectly indicates that part of the antihyperglycaemic activity of THC is due to the release of insulin from the existing  $\beta$ -cells of the pancreas. THC has been shown to potentiate insulin secretion, causing a significant decrease in blood glucose (Pari and Murugan 2005; Murugan and Pari 2006). Despite the fact that THC potentiates insulin secretion, in the present study, we showed that STZ-nicotinamide caused a decrease in the number of insulin-binding sites, and administration of THC increased the number of binding sites.

In the present investigation, treatment with THC led to significant antihyperglycaemic activity. This is probably indicative of the efficacy of THC. In addition, despite the fact

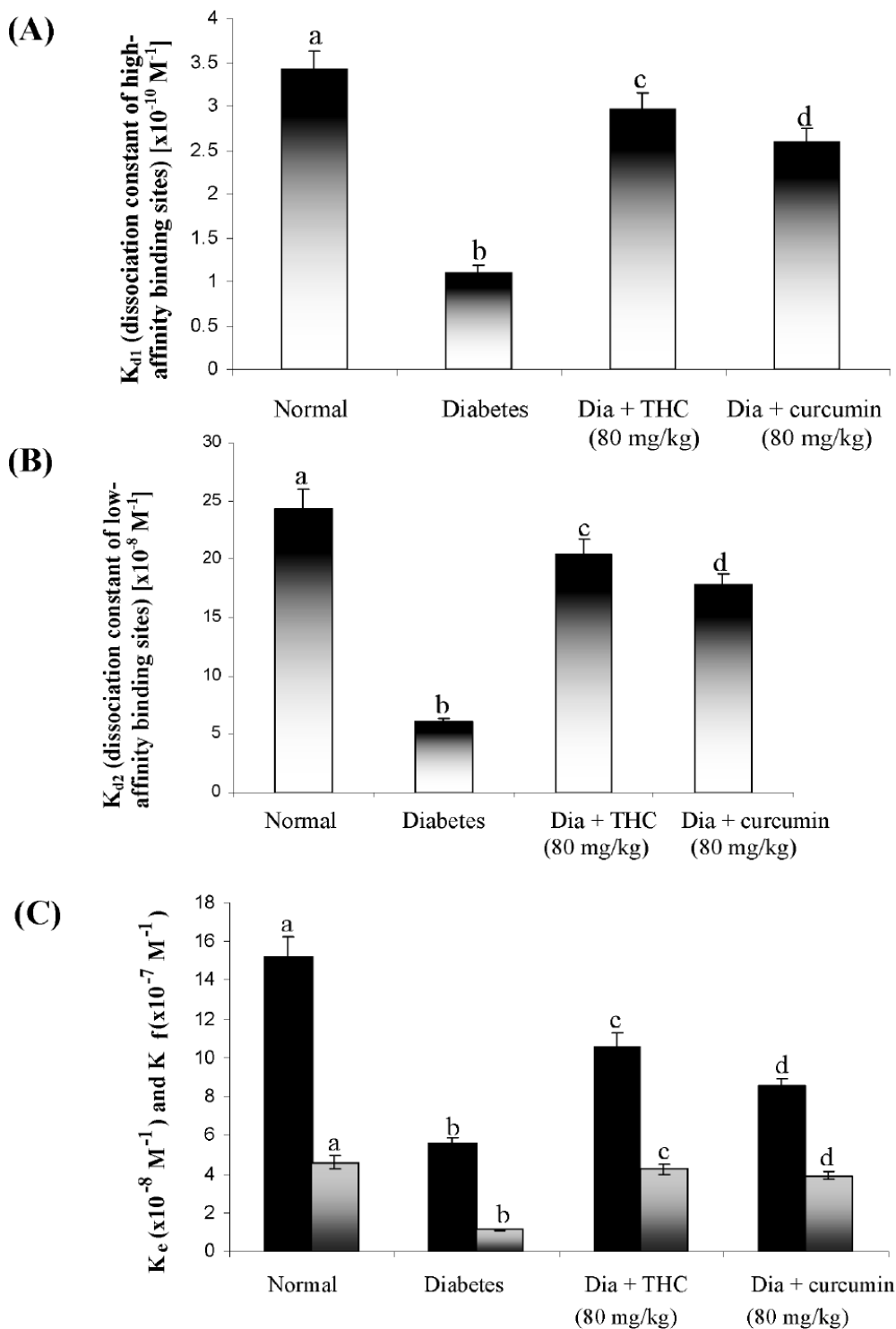
that THC and curcumin stimulate insulin secretion, we have also shown that diabetic rats treated with THC and curcumin showed an increase in the number of insulin-binding sites.

Using erythrocytes as the study tool, we have shown that in rats with STZ-nicotinamide-induced diabetes there is a decrease in the specific binding of insulin to insulin receptors on erythrocytes. This appears to be mainly due to a significant decrease in the receptor concentration per cell and also due to a marginal decrease in the affinity of the receptors to insulin. As insulin levels decrease in diabetic rats, insulin binding to the receptors also decreases (Frank *et al* 1986; Sukhinder *et al* 2001; Olefsky and Reaven 1976).

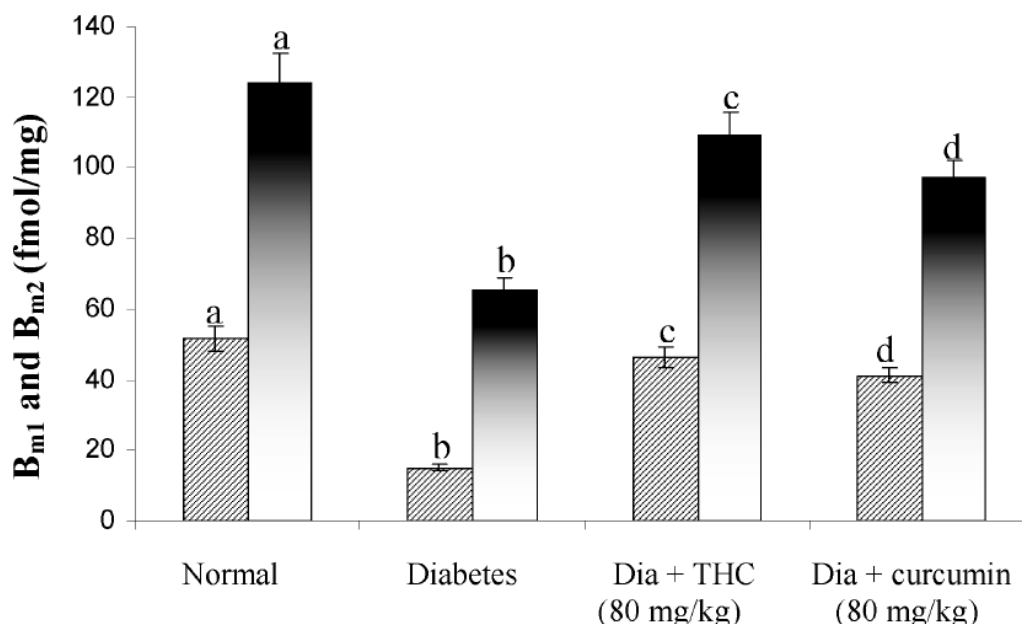
To investigate whether the decrease in insulin binding could be attributed to a decrease in the number of receptor sites per cell, the Scatchard plot for the data was analysed. Using this method of analysis, the X-intercept represents the number of insulin receptor sites per cell. Calculations revealed that there was a decrease in the number of receptor sites per erythrocyte in diabetic rats compared with THC- and curcumin-treated ones. Therefore, the decrease in insulin binding due to the effects of STZ-nicotinamide is primarily the result of a decrease in the number of receptor sites per cell.

The cooperative interactions among receptor sites can be explained in terms of the “negative cooperativity” model. Negative cooperativity is a frequent occurrence in hormone-receptor systems in which there are site-site interactions, resulting in a decrease in the apparent affinity of the receptor for insulin when fractional saturation of the receptor increases. According to this model and calculation of the number of receptor sites using Scatchard analysis, the decreased insulin binding observed was primarily due to a decrease in the number of receptor sites. It can be suggested that the lower number of receptor sites per cell in rats treated with STZ-nicotinamide could be the result of primary alteration in the receptor or was secondary to some other alterations in the integrity of the membrane (generation of free radicals) (Slater 1984). STZ-nicotinamide may damage erythrocytes in the short term with direct effects on membrane structure, membrane fluidity, cross-linking and function. The molecular nature of and site-site interactions between receptor sites may include mechanisms such as intramolecular changes in the tertiary or quaternary structure of the receptor, association and dissociation of the receptor molecules, clustering of receptors in the membrane or phase transitions in the membrane itself. These changes in membrane integrity could be responsible for the decreased number of receptor sites per cell. The resulting membrane dysfunction can impair transport of glucose across the cell membrane resulting in the hyperglycaemia observed.

The contributory effect of any alteration in receptor affinity was evaluated by average affinity. The significant



**Figure 5.** (A)  $K_{d1}$  of high-affinity binding sites profile of normal and experimental rat erythrocytes. Values are given as mean  $\pm$  SD for 6 rats in each group. Values that do not share a common superscript letter differ significantly at  $P < 0.05$  (DMRT). (B) Profile of normal and experimental rats erythrocytes showing  $K_{d2}$  of low-affinity binding sites. Values are given as mean  $\pm$  SD for 6 rats in each group. Values that do not share a common superscript letter differ significantly at  $P < 0.05$  (DMRT). (C)  $K_e$  and  $K_f$  – Affinity constant for empty receptors (■) and affinity constant for filled receptors (▒) of normal and experimental rat erythrocytes. Values are given as mean  $\pm$  SD for 6 rats in each group. Values that do not share a common superscript letter differ significantly at  $P < 0.05$  (DMRT). Dia, diabetes; THC, tetrahydrocurcumin.



**Figure 6.** B<sub>m1</sub> and B<sub>m2</sub>. Number of high- (▨) and low (■)-affinity binding sites of normal and experimental rat erythrocytes. Values are given as mean  $\pm$  SD for 6 rats in each group. Values that do not share a common superscript letter differ significantly at  $P < 0.05$  (DMRT). Dia, diabetes; THC, tetrahydrocurcumin.

decrease in the empty-site affinity for erythrocytes supports the concept of alterations in both receptor affinity and receptor sites, each contributing to decreased insulin binding. Some of the insulin receptors on the cell membranes of erythrocytes from diabetic rats could have been affected by the cytotoxic effect of STZ and become desensitized as a consequence of increased glucose concentration. Desensitization is associated with a total absence of the effect of insulin, despite the presence of insulin receptors. The mechanism of changing receptor affinity and desensitization can be explained by several possibilities. First, fluidity may be an important factor in modulating insulin binding and action. Second, the insulin receptor may be covalently associated with another protein that modulates receptor affinity (Ashokkumar *et al* 2006). It is therefore a possibility that overproduction of free radicals due to lipid peroxidation may alter the interaction of insulin with its receptors, thus affecting the ability of insulin to differentially regulate its receptor and this regulator protein (Harmon *et al* 1980). Free radicals, especially increased generation of NO in the diabetic state, may also affect the formation of the insulin-receptor complex. A third possibility is that the receptor may undergo some form of post-translational modification that alters binding and signal transmission properties (Kasuga *et al* 1982). This post-translational modification could involve a change in the redox state of the receptor. The insulin receptor is composed of major subunits linked by disulphide bonds to various oligomeric forms. Reduction of the oxidized

forms of the receptors could modify their affinity for insulin (Massague and Czech 1982).

The advantage of using circulating erythrocytes for investigating the receptor status in human and animals is that they are more easily accessible than cells of primary insulin target organs, such as adipocytes and muscles (Okada 1981; Tomasevic *et al* 2003). Oral hypoglycaemic agents have been reported to improve insulin receptor status (Ragoobirsingh *et al* 1990; Proks *et al* 2002; Patane *et al* 2000). In the present study, the administration of THC and curcumin increased the plasma insulin levels and improved insulin binding to isolate erythrocyte insulin receptors. Multiple factors may be responsible for such a rapid improvement in insulin action *in vivo*. Therefore, erythrocytes represent a more uniform population of cells capable of the same receptor-mediated function as adipocytes, providing a clear reflection of the insulin receptor status of target tissues.

## 5. Conclusion

In conclusion, the present study showed that oral treatment with THC and curcumin improved the erythrocyte membrane insulin-binding sites with a concomitant increase in plasma insulin. The molecular basis for each event that occurs after the binding of insulin to its receptor remains to be examined.

## References

- Aggarwal B, Kumar A and Bharti A 2003 Anticancer potential of curcumin: preclinical and clinical studies; *Anticancer Res.* **23** 363–398
- Ashokkumar N, Pari L and Rao C H A 2006 Effect of N-benzoyl-D-phenylalanine and metformin on insulin receptors in neonatal streptozotocin-induced diabetic rats: studies on insulin binding to erythrocytes; *Arch. Physiol. Biochem.* **112** 1174–112181
- Babu P and Srinivasan K 1997 Hypolipidemic action of curcumin, the active principle of turmeric *Curcuma longa* in STZ induced diabetic rats; *Mol. Cell. Biochem.* **166** 169–175
- Babu P and Srinivasan K 1998 Amelioration of renal lesions associated with diabetes by dietary curcumin in STZ diabetic rats; *Mol. Cell. Biochem.* **181** 87–96
- De Pirro R, Rucco A and Lauro R 1980 Erythrocyte insulin receptors in non-insulin dependent diabetes mellitus; *Diabetes* **29** 96–99
- Dons R F, Ryan J, Gorden P and Rodbard W H 1981 Erythrocyte and monocyte insulin binding in man: a comparative analysis in normal and disease states; *Diabetes* **30** 896–902
- Duncan B D 1957 Multiple ranges tests for correlated and heteroscedastic means; *Biometrics* **13** 359–364
- Frank H J, Pardridge W M, Vokes J T, Vinters H V and Morris W L 1986 Insulin binding to the blood-brain barrier in the STZ diabetic rat; *J. Neurochem.* **47** 405–411
- Gambhir K K, Archer J A and Bradley C J 1978 Characteristics of human erythrocyte insulin receptors; *Diabetes* **28** 701–708
- Garcia-Alloza M, Borrelli L A, Rozkalne A, Hyman B T and Bacskai B J 2007 Curcumin labels amyloid pathology in vivo, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model; *J. Neurochem.* **102** 1021095–10210104
- Grigorescu F, White M F and Kahn C R 1983 Insulin binding and insulin-dependent phosphorylation of the insulin receptor solubilized from human erythrocytes; *J. Biol. Chem.* **258** 13708–13716
- Harmon J T, Kahn C R, Kempner E S and Schlegel W 1980 Characterization of the insulin receptor in its membrane environment by radiation inactivation; *J. Biol. Chem.* **255** 3412–3419
- Insel J R, Kolterman O G, Saekow M and Olefsky J M 1980 Short-term regulation of insulin receptor affinity in man; *Diabetes* **29** 132–139
- Kasuga M, Zick Y, Blithe D L, Crettaz M and Kahn C R 1982 Insulin stimulates tyrosine phosphorylation of the insulin receptor in a cell-free system; *Nature* **298** 667–669
- Kolterman O G, Gray R S, Griffin J, Burstein J, Sinse J, Scarlett J A and Olefsky J M 1981 Receptor and post-receptor defects contribute to the insulin resistance in non-insulin dependent diabetes mellitus; *J. Clin. Invest.* **68** 957–969
- Lim J K and Lin-Shiau S Y 2001 Mechanisms of cancer chemoprevention by curcumin; *Proc. Natl. Sci. Counc. Repub. China* **25** 59–66
- Lott J A and Turner K 1975 Evaluation of Trinder's glucose oxidase method for measuring glucose in serum and urine; *Clin. Chem.* **21/12** 1754–1760
- Mahesh T, Balasubashini M and Menon V 2005 Effect of photo-irradiated curcumin treatment against oxidative stress in STZ-induced diabetic rats; *J. Med. Food* **8** 251–255
- Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M and Ribes G 1998 Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide; *Diabetes* **47** 224–229
- Massague J and Czech M P 1982 Role of disulphides in the subunit structure of the insulin receptor; *J. Biol. Chem.* **257** 6729–6738
- McElduff A and Eastman C J 1981 The erythrocyte insulin receptor; *Aust. J. Exp. Biol. Med. Sci.* **59** 439–448
- Murugan P and Pari L 2006a Antioxidant effect of tetrahydrocurcumin in STZ–nicotinamide induced diabetic rats; *Life Sci.* **79** 1720–1728
- Murugan P and Pari L 2006b Effect of tetrahydrocurcumin on plasma antioxidants in streptozotocin–nicotinamide induced experimental diabetes; *J. Basic Clin. Physiol. Pharmacol.* **17** 231–244
- Murugan P and Pari L 2006 Effect of tetrahydrocurcumin on lipid peroxidation and lipids in streptozotocin – nicotinamide-induced diabetic rats; *Basic Clin. Pharmacol. Toxicol.* **99** 122–127
- Murugan P and Pari L 2007 Protective role of tetrahydrocurcumin on changes in the fatty acid composition in streptozotocin–nicotinamide induced type 2 diabetic rats; *J. Appl. Biomed.* **5** 31–38
- Naito M, Wu X, Normura H, Kodama M, Kato Y and Osawa T 2002 The protective effect of THC on oxidative stress in cholesterol-fed rabbits; *J. Atheroscler. Thromb.* **9** 243–250
- Nishiyama T, Mae T, Kishida H, Tsukagawa M, Mimaki Y, Kuroda M, Sashida Y, Takahashi K, Kawada T, Nakagawa K and Kitahara M 2005 Curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa* L) suppress and increase in blood glucose level in type 2 diabetic KK-Ay mice; *J. Agric. Food Chem.* **53** 959–963
- Novelli M, Fabregat M E, Fernandez-Alvarez J, Gomis R and Masiello P 2001 Metabolic and functional studies on isolated islets in a new rat model of type 2 diabetes; *Mol. Cell. Endocrinol.* **175** 57–66
- Okada K, Wangpoengtrakul C, Tanaka T, Toyokuni S, Uchida K and Osawa T 2001 Curcumin and especially THC ameliorate oxidative stress-induced renal injury in mice; *J. Nutr.* **31** 2090–2095
- Okada Y 1981 Increased insulin binding to erythrocytes in chronic liver disease; *Acta Med. Okayama* **35** 155–164
- Olefsky J M and Kolterman O G 1981 Mechanisms of insulin resistance in obesity and non-insulin dependent (Type II) diabetes; *Am. J. Med.* **70** 151–168
- Olefsky J M and Reaven G M 1976 Effects of sulphonylurea therapy on insulin binding to mononuclear leukocytes of diabetic patients; *Am. J. Med.* **60** 89–95
- Pari L, Latha M and Appa Rao C 2004 Effect of *Scoparia dulcis* extract on insulin receptors in streptozotocin induced diabetic rats: studies on insulin binding to erythrocytes; *J. Basic Clin. Physiol. Pharmacol.* **15** 223–240
- Pari L and Murugan P 2004 Protective role of THC against erythromycin estolate induced hepatotoxicity; *Pharmacol. Res.* **49** 481–486

- Pari L and Murugan P 2005 Effect of THC on blood glucose, plasma insulin and hepatic key enzymes in STZ induced diabetic rats; *J. Basic Clin. Physiol. Pharmacol.* **16** 257–274
- Pari L and Murugan P 2006 Changes in glycoprotein components in streptozotocin – nicotinamide induced type 2 diabetes: influence of tetrahydrocurcumin from *Curcuma longa*; *Plant Food Hum. Nut.* DOI: 10.1007/s11130-006-0037-1
- Pollet R J, Haase B A and Standaert M L 1981 Characterization of detergent-solubilized membrane proteins. Hydrodynamic and sedimentation equilibrium properties of the insulin receptor of the cultured human lymphoblastoid cell; *J. Biol. Chem.* **256** 12118–12126
- Patane G, Piro S, Anello M, Rabuazzo A M, Vigneri R, Purrello F 2000 Exposure to glibenclamide increases rat  $\beta$ -cells sensitivity to glucose; *Br. J. Pharmacol.* **129** 887–892
- Proks P, Reimann F, Green N, Gribble F and Ashcroft F M 2002 Sulfonylurea stimulation of insulin secretion; *Diabetes* **51** S368–S376
- Ragoobirsingh D, Robinson H M and Morrison E Y 1990 Insulin receptor studies of erythrocytes and mononuclear leucocytes in phasic insulin diabetes mellitus; *West Indian Med. J.* **39** 144–147
- Reusch J E 1998 Focus on insulin resistance in type-2 diabetes: therapeutic implications; *Diabetes Educator* **24** 188–193
- Sajithlal G, Chithra P and Chandrakasan G 1998 Effect of curcumin on the advanced glycation and cross-linking of collagen in diabetic rats; *Biochem. Pharmacol.* **56** 1607–1614
- Scatchard G 1949 The attraction of proteins for small molecules and ions; *Ann. NY Acad. Sci.* **51** 660–672
- Sidhu G, Mani H, Gaddipati J, Singh A, Seth P, Banaudha K, Patnaik G and Maheshwari R 1999 Curcumin enhances wound healing in STZ induced diabetic rats and genetically diabetic mice; *Wound Rep. Regen.* **7** 362–374
- Slater T F 1984 Free radical mechanisms in tissue injury; *Biochem. J.* **22** 1–15
- Sugiyama Y, Kawakishi S and Osawa T 1996 Involvement of the  $\alpha$ -diketone moiety in the antioxidant mechanism of tetrahydrocurcuminoids; *Biochem. Pharmacol.* **52** 519–525
- Sukhinder K, Cheema M and Clandinin T 2001 Diet and diabetes induced change in insulin binding to the nuclear membrane in spontaneously diabetic rats is associated with change in the fatty acid composition of phosphatidylinositol; *J. Nutr. Biochem.* **12** 213–218
- Suryanarayana P, Saraswat M, Mrudula T, Krishna P, Krishnaswamy K and Reddy G 2005 Curcumin and turmeric delay STZ-induced diabetic cataract in rats; *Invest. Ophthalmol. Vis. Sci.* **46** 2092–2099
- Susheela L, Ramachandran A, Mohan V, Sheeja and Viswanathan M 1987 Erythrocyte insulin receptor abnormalities; *J. Assoc. Physicians India* **35** 337–339
- Tomasevic N, Nikoli M, Klappe K, Hoekstra D and Niketi V 2003 Insulin-induced lipid binding to hemoglobin; *J. Sereb. Chem. Soc.* **68** 25–33
- Ward G M and Harrison L C 1986 Structure of the human erythrocyte insulin receptor; *Diabetes* **35** 101–105

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