Hyperglycemia Alters Maternal-Fetal Transport Kinetics of Manganese, Chromium and Vanadium in Diabetic Model Placental Lobule In Vitro: Implications for Diabetes Mellitus

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Diabetes mellitus in pregnancy well known to contribute to increased maternal and neonatal mortality as well as morbidity

Many Reports ***(Nandakumaran et al, 1999, 2002)*** **have also implicated altered status of essential trace elements in diabetic state to be partly responsible for inducing congenital malformations in in infants of diabetic women as well** ***(Eriksson& Borg 1991 ; 1993 ; Willhoite et al, 1993)***

Importance of a variety of trace elements in maintenance of health ***(Mertz W , 1981 ; Peerebom 1985 ; Gibson 1989)*** **has been highlighted by several international research groups.**

More recently, many research groups have emphasized the importance of trace elements such ac chromium, manganese and vanadium both animal and human studies

We had reported ***(Nandakumaran et al, 2004, 2005, 2006)*** **altered maternal-fetal disposition of some essential trace elements in human diabetic pregnancies as well as in experimentally-induced diabetic rats as well**

More recently, many research groups have emphasized the importance of trace elements such as chromium and vanadium in both animal and human studies

Chromium, has been reported to be an essential trace element for human nutrition, required for normal carbohydrate and lipid metabolism ***(Mertz 1993 ; Anderson, 1993).***

Severe signs of chromium deficiency such as nerve and brain disorders have been reported to be reversed by supplemental Cr in patients on total parenteral nutrition ***(Jeejeebhoy 1977 ; Brown et al, 1986).***

Further, chromium supplementation has been shown to have beneficial effects on people with varying degrees of glucose intolerance
(Anderson, 1998)

Another trace element Vanadium, has been proposed to be one of the nutritionally essential mineral elements for human health ***(Nielson FH 1997 ; Barceloux 1999).***

Vanadium has been shown to have an insulin-like effect ***(Dunai &Saminathan, 1997 ; Crans 2000)*** **and has been reported to be useful in overcoming insulin resistance** ***(Cusi et al, 2001 ; Goldfine et al,1995)*** **in humans.**

However data on maternal-fetal disposition and transport and disposition of above essential trace elements in human placenta in control as well as diabetic pregnancies have not been explored by any research group so far

Considering the relatively high incidence of diabetes mellitus in obstetric population all over the world, we thought it interesting to investigate this crucial problem in a specially designed diabetic human placental model

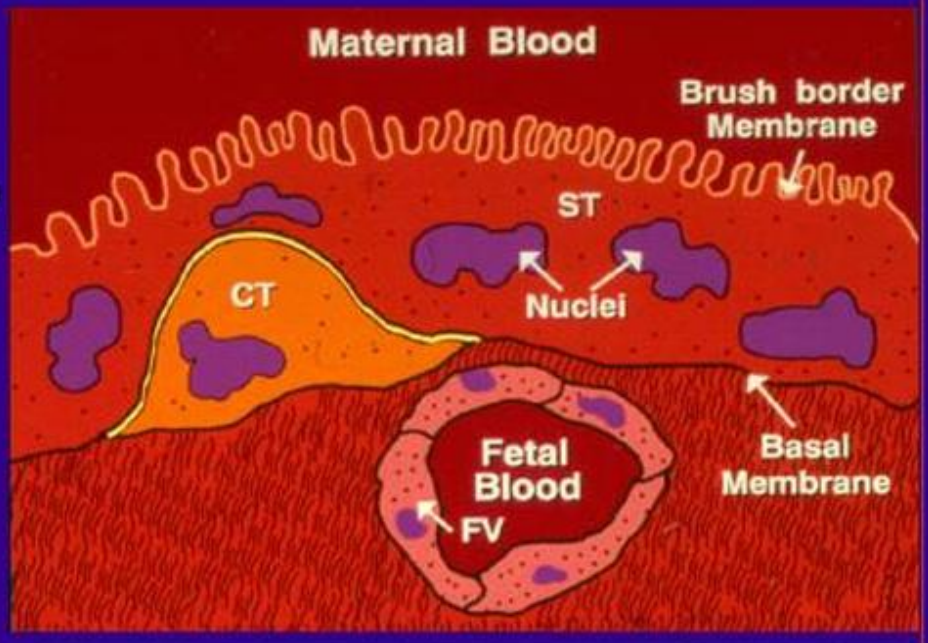
Mn is an esssential co-factor for enzymes hexokinase, superoxide dismutase and xanthine oxidase and the trace element has been shown to scavenge free radicals in vitro in animals. However, no detailed study has been done, to our knowledge on the association between Mn level and diabetes in pregnancy in humans.

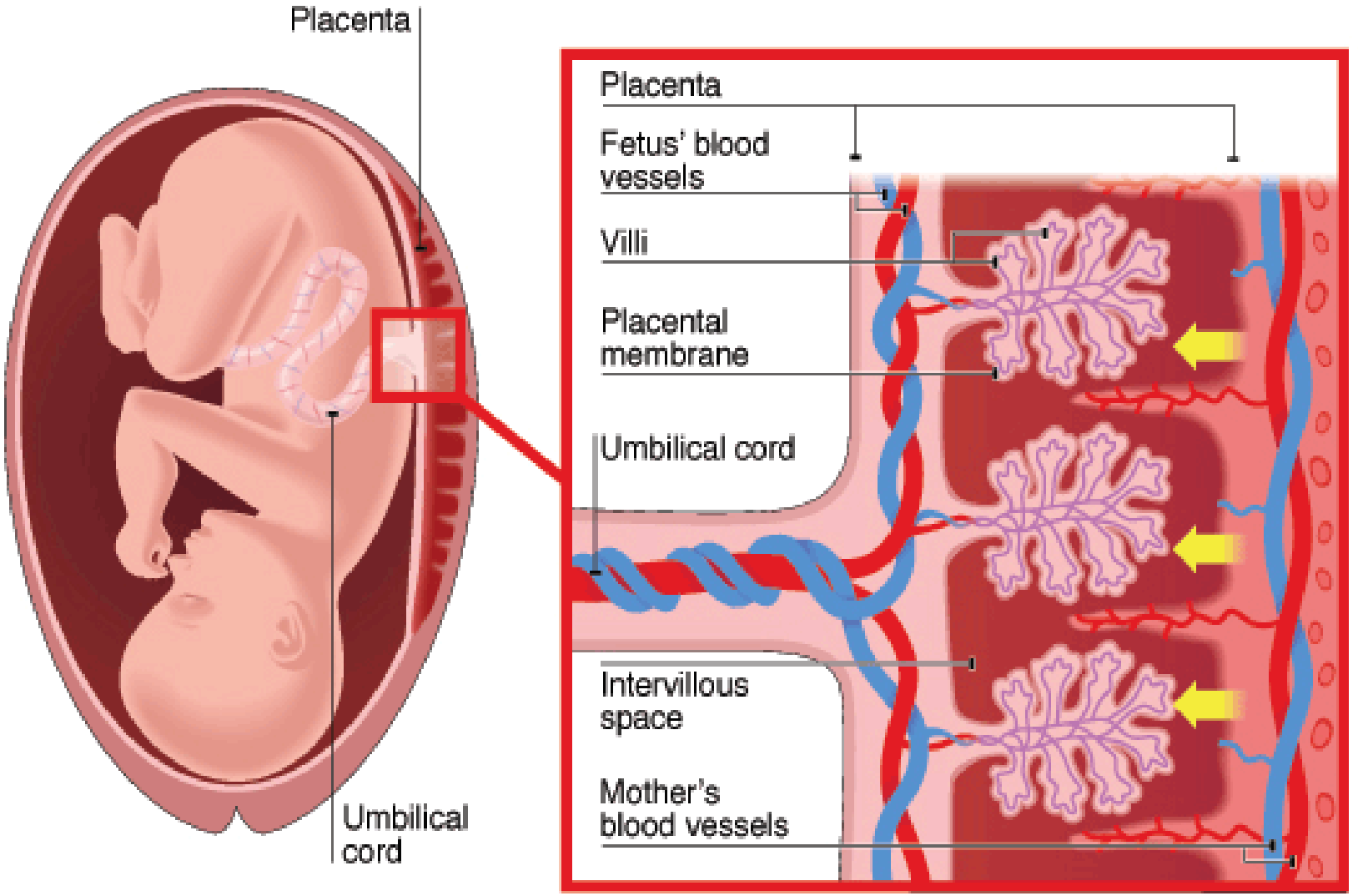
To Assess the probable impact on the developing fetus which could possibly help the obstetrician and neonatologist in better management of the diabetic mother and her offspring.

MATERIAL & METHODS

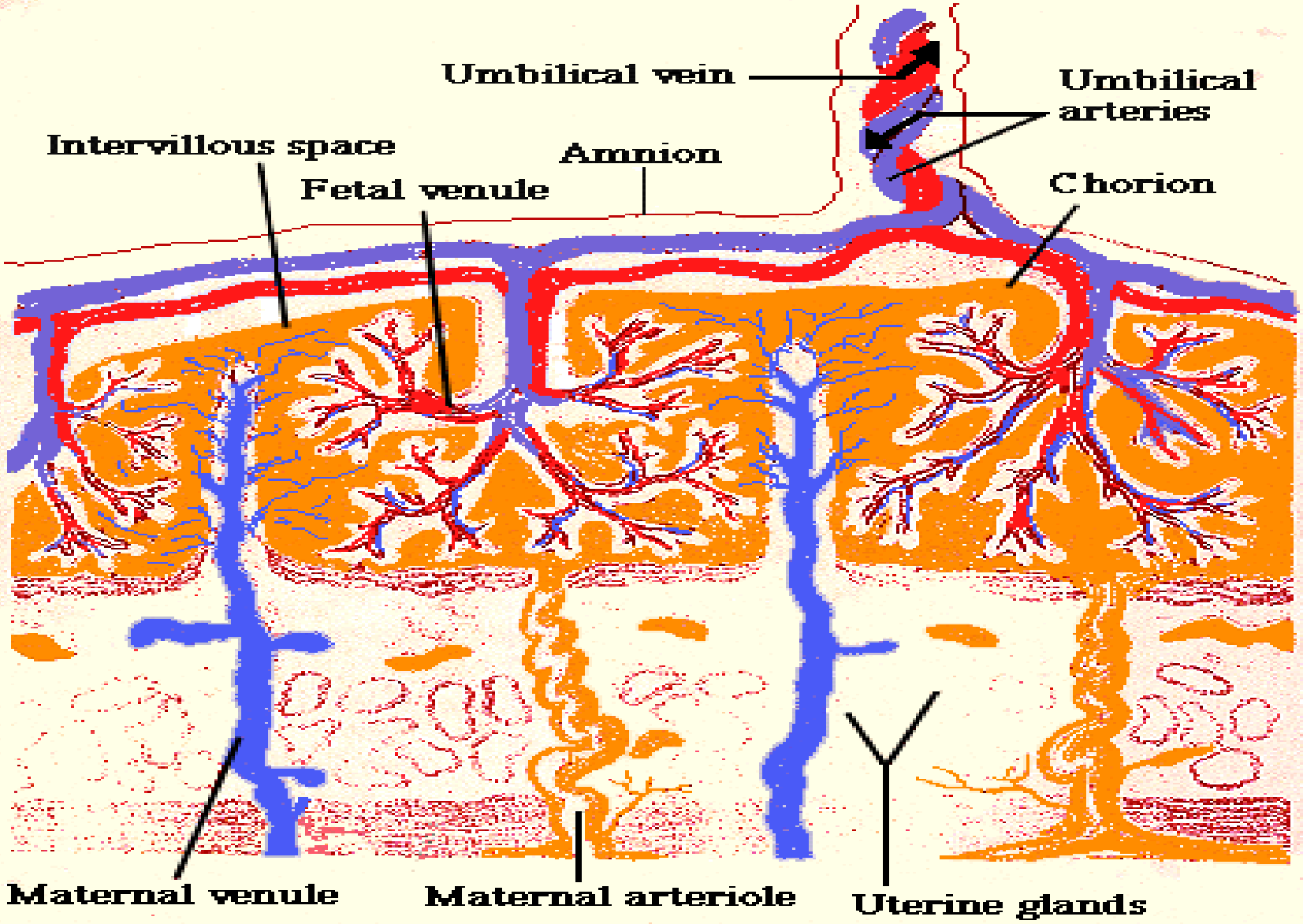
Human Placentae were collected immediately after delivery and transported to our laboratory and suitable isolated lobule perfused within 60 minutes using an in vitro perfusion system.

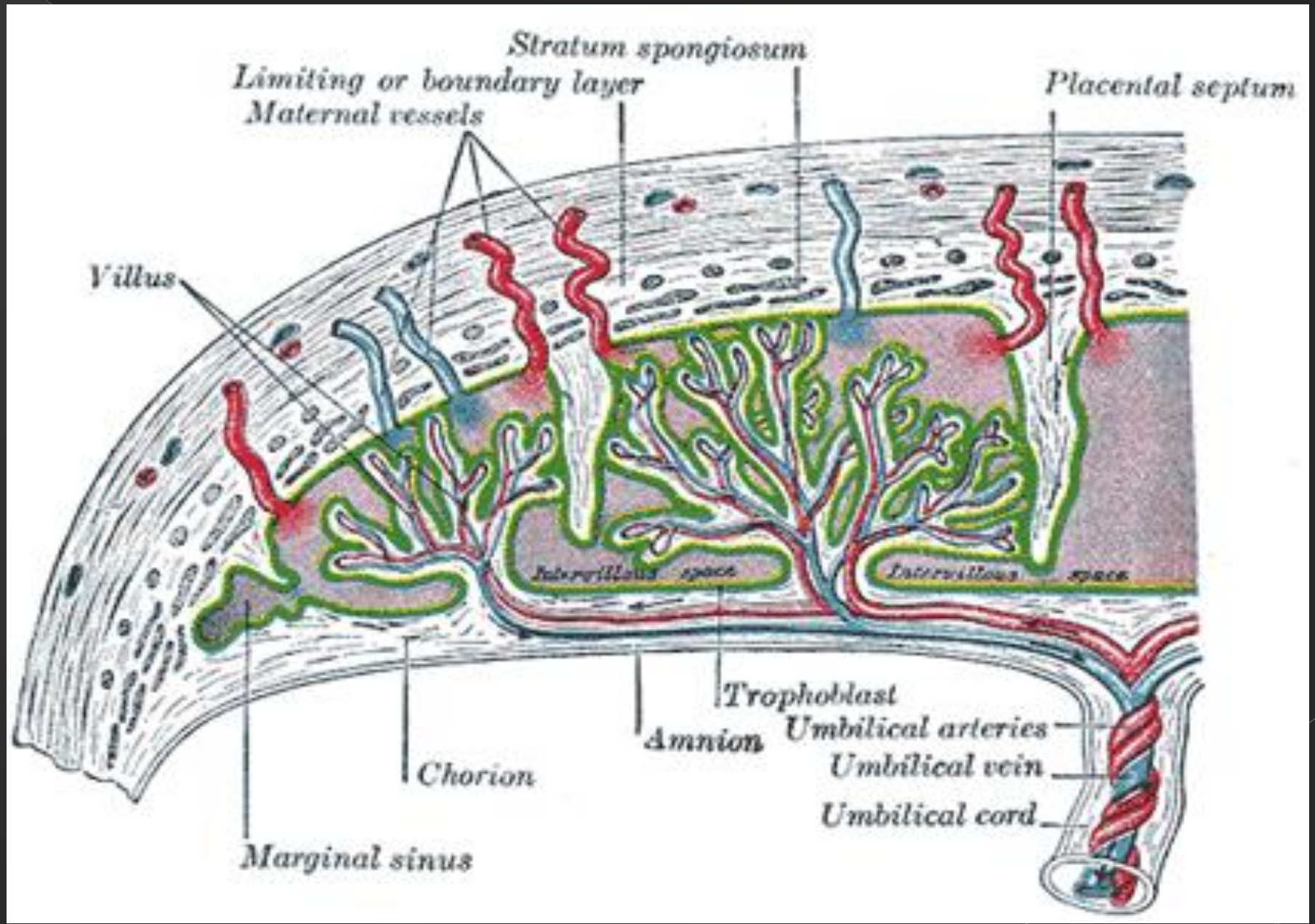
Human Placenta

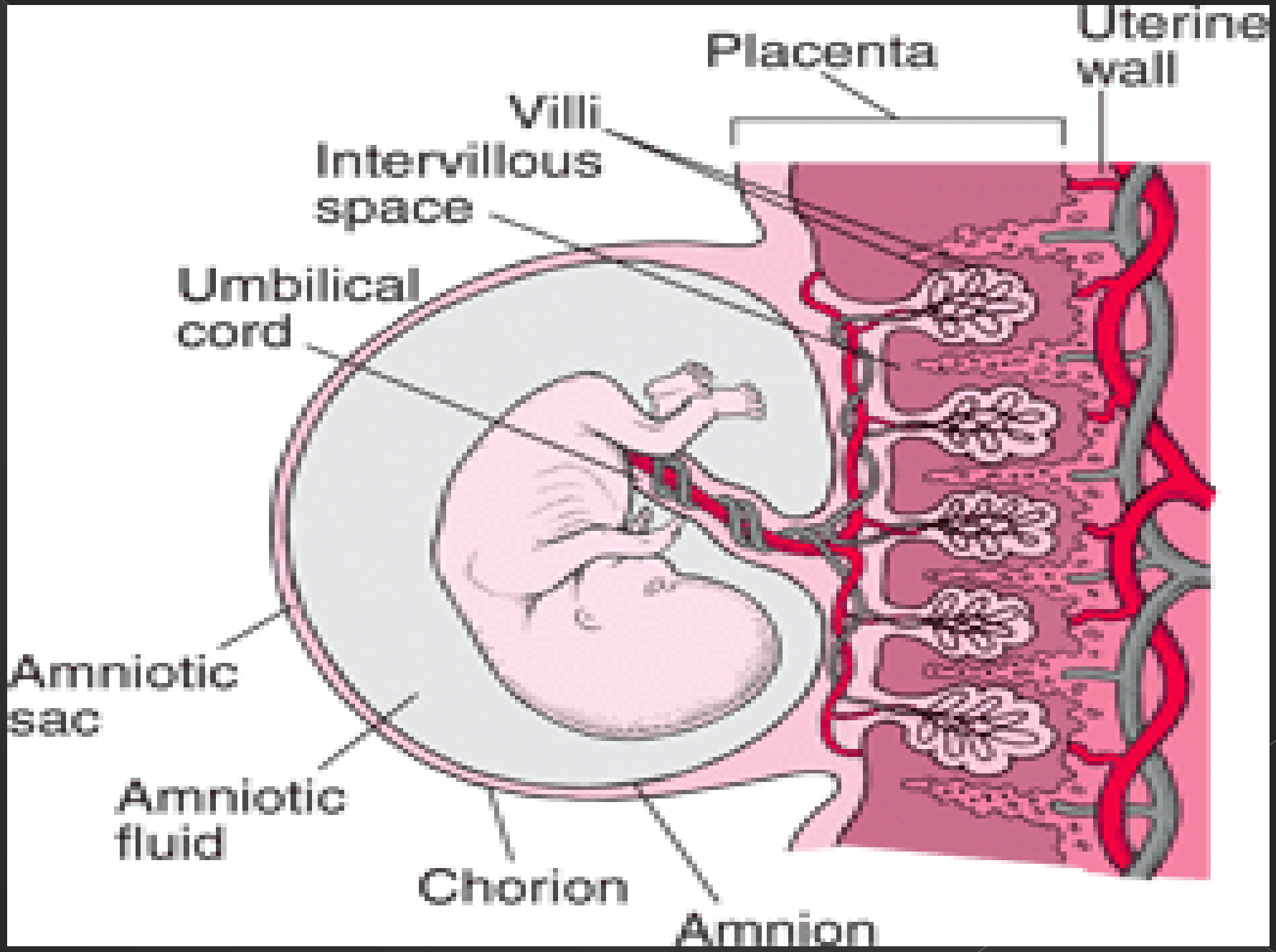



Placenta
Umbilical cord
Placenta
Fetus' blood vessels
Villi
Placental membrane
Umbilical cord
Intervillous space
Mother's blood vessels

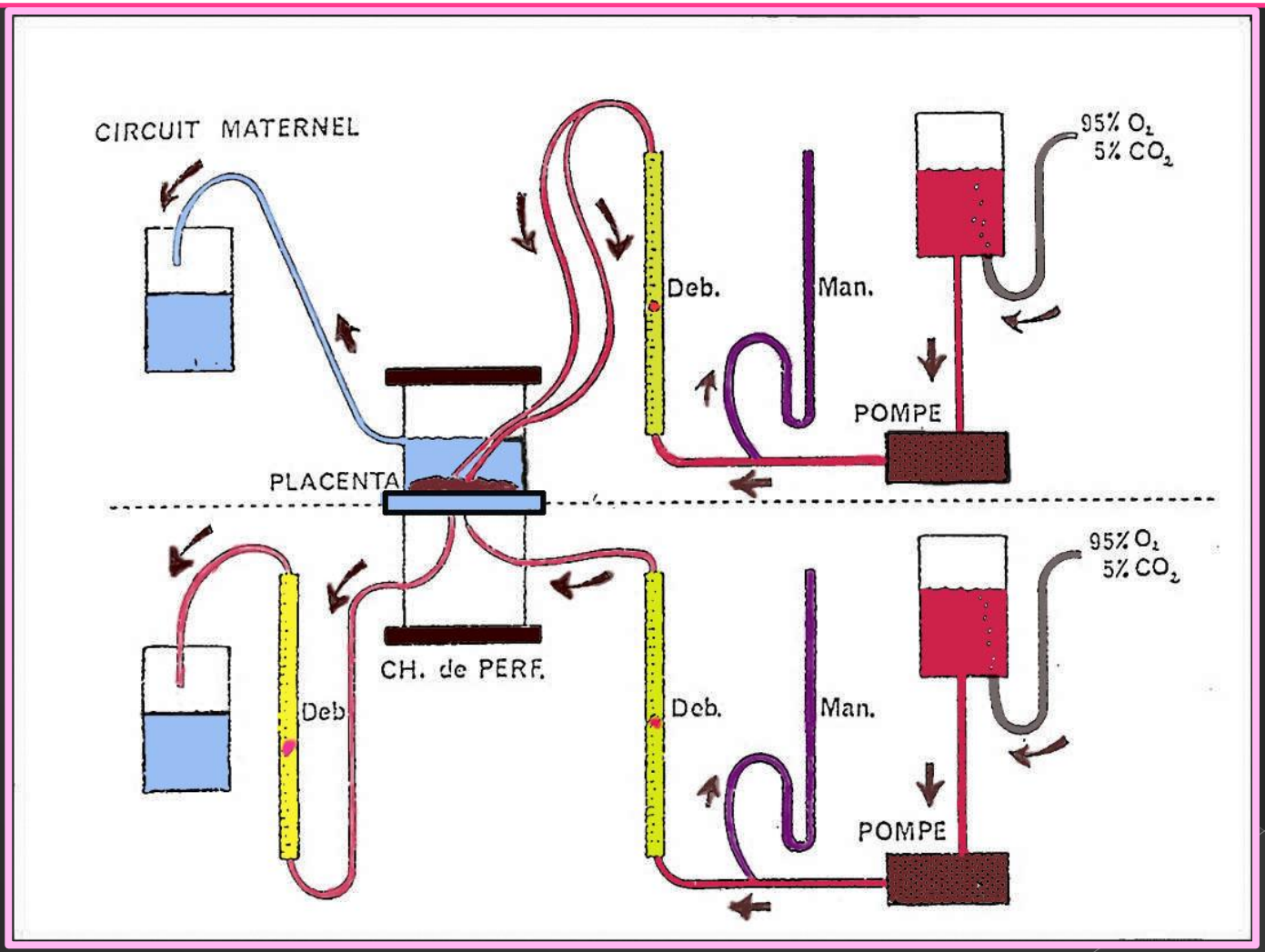
Placenta: fetal and maternal blood vessels




Stratum spongiosum
Limiting or boundary layer
Maternal vessels
Placental septum
Villus
Trophoblast
Umbilical arteries
Amnion
Umbilical vein
Chorion
Umbilical cord
Marginal sinus


Placenta
Uterine wall
Villi
Intervillous space
Umbilical cord
Amniotic sac
Amniotic fluid
Chorion
Amnion

Schematic Representation of Perfusion Assembly



Control perfusions was done, as per the technique described ***(Nandakumaran et al, 1981, 1984, 1999, 2002, 2006)*** **using NCTC Medium containing euglycemic load (1 g/L) and containing physiological concentrations of amino acids and free fatty acids, albumin, etc**

Perfusion of isolated human placental lobules from diabetic and uncomplicated control pregnancies was performed as per the technique described ***(Nandakumaran et al, 1981, 1984, 1999, 2002, 2006)***

Control perfusions was done using NCTC Medium containing euglycemic load (1 g/L) and conatining physiological concentrations of amino acids and free fatty acids and protein,etc

Transport kinetics of trace elements were explored in in separate series of experiments, using a diabetic model placentae with hyperglycemia of 200 g/L , mimicking a moderate hyperglycemic state

Circulation of the perfusate was effected by Harvard digital pump and fetal and maternal flow rates was assessed by BROOKS R-215 flowmeters. Fetal and maternal flow rates were maintained within physiological range and pressure in both the circuits were monitored by mercury manometers.

After a wash-out phase of 10 minutes. trace elements (Cr & Mn& V) at concentrations twice the normal concentrations reported in in vivo state, were injected as a 100 ul bolus along with antipyrine (1g/L) as an internal reference marker.

And perfusate samples were collected every 15 seconds from fetal and maternal circuits after a lag period of 1 minute, for a period of 5 minutes.

Viability of perfusions was assessed by assessing oxygen consumption of perfused tissue as well as by assessing absence of lactic dehydrogenase (LDH) enzyme in the perfusate samples before and after perfusion.

- **Diabetic Model Hyperglycemic Perfusions were done separately in the case of above Trace Elements (Cr,Mn&V) by increasing glucose concentration to twice the normal (2 g/L) in maternal perfusate and perfusate sample s collected from maternal and fteal venous outflow as described earlier**

Concentration of trace elements in perfusate samples and injectate were determined by atomic absorption spectrophotometry ***(Kosenko 1964 ; Krachler et al 1996, 1999 ; Walter et al, 1991)*** **.**

Antipyrine, reference marker concentration in various samples was assessed by a colorimetric technique ***(Nandakumaran et al, 1981, Brodie et al, 1949)***

Maternal-fetal transport parameters and kinetics were assessed by using the following parameters.

Differential transport rates of test and reference substances were computed as described.

(Nandakumaran et al, 1991 ; 1999, 2002).

Briefly, the fraction of trace element in the fetal perfusate was plotted cumulatively as a function of perfusion time and the time required in minutes for 10, 25, 50, 75 and 90% of substance efflux in the fetal vein calculated using the plotted curve.

Transport of various trace elements studied were expressed as transport rate indices of different efflux fractions, as ratio of corresponding reference marker transport rates.

$$\text{Efflux Fraction} = \frac{\text{EFS}}{\text{TEFV5}}$$

Where EFS=Concentration of the element studied in fetal venous sample;TEFV5=Total inorganic element concentration of the element studied in fetal venous outflow for period of 5 min.

Transport fraction (TF) of substances studied was calculated as per the following formula ***(Nandakumaran et al, 1984, 2002, 2005, 2009)***

$$TF = \frac{\text{Total study/ref. substance conc. in the fetal vein}}{\text{Total study/ref. substance load in the injected bolus}}$$

A TF index of study substance was computed by expressing the TF value as a ratio of that of the reference.

To assess the transport rate of the trace element and reference marker were assessed by plotting their concentrations as a function of perfusion time

(Nandakumaran et al, 1999, 2001, 2002, 2008).

Area under the curve (AUC) of substances studied was computed using trapezoid rule ***(Rey, Nandakumaran et al,1984, Nandakumaran et al, 1999, 2002)*** **assuming a two-compartment model.**

$$AUC = \sum_{i=1}^{n} \frac{[C(1+1)+ C(I)] \times [t(i+1)-t(i)]}{2} + \frac{C(n)}{Kel}$$

Parameters as clearance, Kel (elimination constant), Tmax (time of maximum response), absorption rate and elimination rate was determined using a computer programme.

Calculations are based on specialized software such as PK2 Solutions (USA) or using IMSL FORTRAN SUBROUTINE software or using appropriate software (Pharmaco-kinetic Software Package, PK2 Solutions, USA).

To minimize experimental artifacts and to minimize inter-experimental variability, kinetic indices of trace elements studied was calculated, expressing the parameter value of the study substance as ratio of corresponding reference.

Data Analysis

Data are presented as Means+ s.e.m or and Statistical Analysis of Data done using SPSS and other appropriate statistical software.

Pharmacokinetic analysis of data are done by trapezoid rule and using the formula indicated earlier

Appropriate statistical package such as PK2 Solutions, etc. was used to verify accuracy of our computations

Tests for significance was done using Student's t-test, Fischer Exact Test, Analysis of Variance, Analysis of Co-variance or other appropriate tests.

Patient details and characteristics

File no.	Age	Weight (kg)	Height (cm)	Parity	Gestation age (weeks+ days)	Apgar score 1'5'	New born weight (kg)	Sex	Placental weight (grams)
1	36	85	164	P0+0+0+0	37+0	7/9	3.440	F	790
2	25	78	158	P0+0+0+0	39+0	8/9	2.47	M	620
3	35	68	152	P1+0+0+1	36+5	8/9	2.95	F	820
4	33	90	165	P2+0+0+2	37+0	9/9	2.90	F	640
5	43	78.5	160	P5+2+0+7	38+0	9/9	3.55	F	670
6	32	80.5	162	P1+0+0+1	38+0	9/9	2.99	M	640
7	25	75.6	163	P4+0+0+4	39+0	8/9	3.10	M	650
8	16	70.2	163	P0+0+0+0	41+2	8/9	3.14	F	530
9	28	74	165	P2+0+0+2	38+3	9/9	2.88	M	575
10	28	89	166	P1+0+0+1	39+6	8/9	3.53	M	520
11	33	84	168	P3+0+0+3	37+3	8/9	3.120	F	650
12	29	90	168	P0+0+0+0	37+2	8/9	3.48	M	680
Mean ±SEM	30.93±1.8	79.85±2.2	163.2±1.18	P1.6+0+0+1.7	38+2	8/9	3.2±0.11		655±38.2

VANADIUM 1 G/L

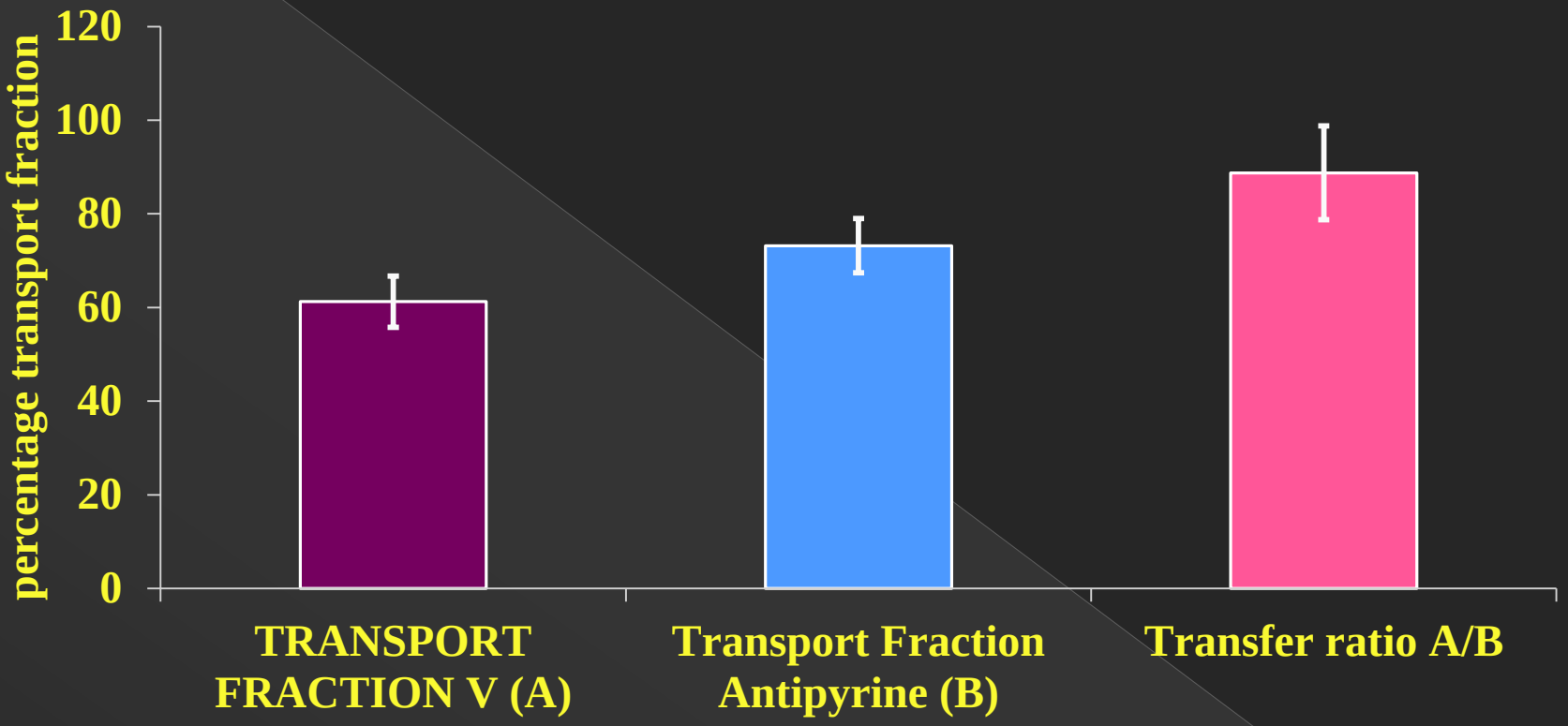
Differential transport rate of antipyrine and vanadium in normoglycemia

	10	25	50	75	90	Cotyledon wt
antipyrine	0.611± 0.06	1.317 ± 0.05	2.494± 0.04	3.672 ± 0.03	4.37 ± 0.03	31.67 ± 1.8
vanadium	0.572 ± 0.04	1.292 ± 0.04	2.491 ±0.05	3.688 ±0.07	4.40 ±0.08	31.67 ± 1.8
Significance	NS	NS	NS	NS	NS	

Pharmacokinetic parameters of Antipyrine and vanadium in normoglycemia

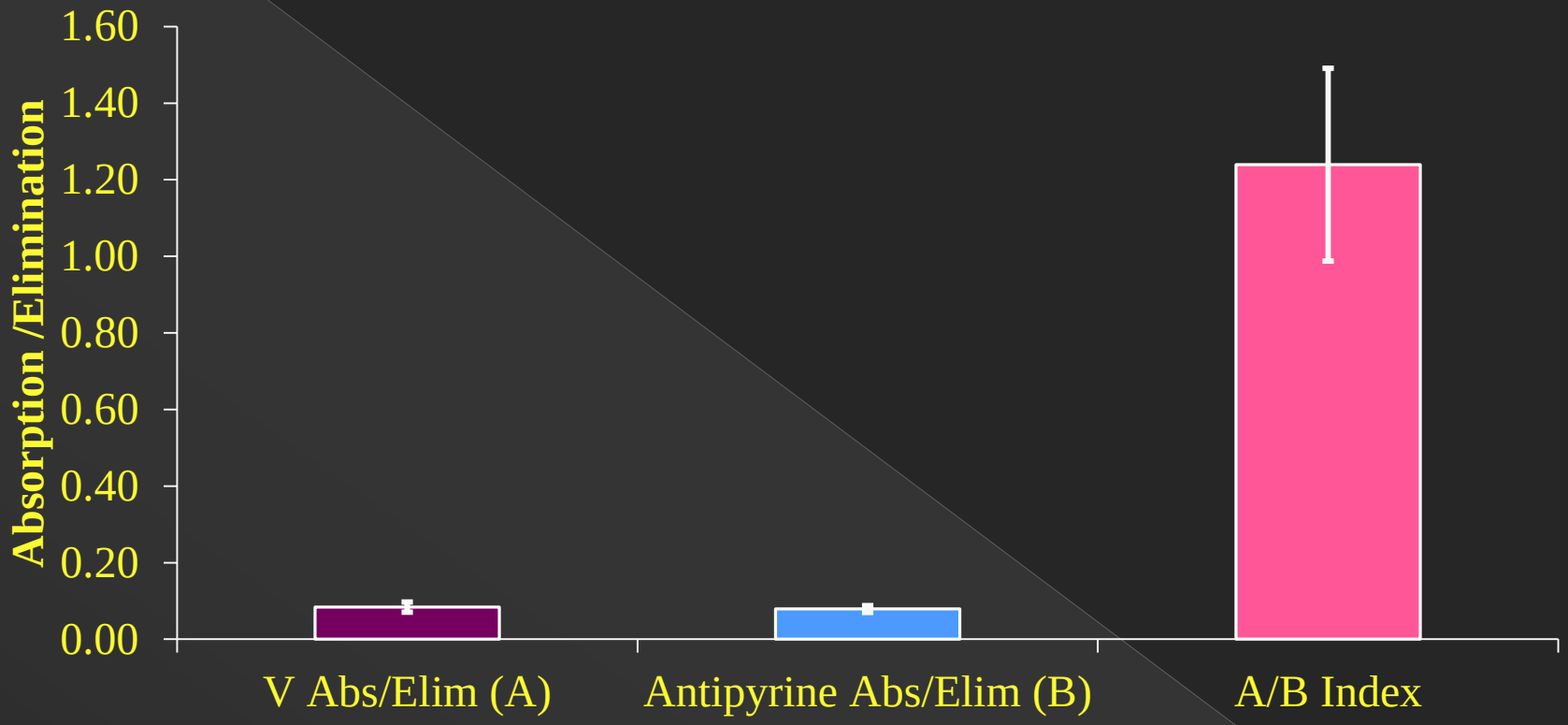
	AUC (ug-sec/l)	clearance (l/sec)*10^7	$kelsec^{-1}$	Tmax (sec)	absorption rate ug/sec *10^7	Elimination rate ug/sec
Antipyrine	4853.49 ± 2716.76	0.006 ± 0.001	0.27 ± 0.01	150.0 ± 47.86	0.094 ± 0.05	1.24 ± 0.72
VANADIUM	8774.7 ± 840.02	0.000011 ±0.0000	0.279 ± 0.0062	150.0 ± 13.8	0.00027 ± 0.00004	0.00350 ± 0.0003
Significance	P<0.0001	P<0.0001	NS	NS	P=0.01	P<0.0001

Transport fraction of vanadium and Antipyrine with 1g/l glucose



Transport fraction of vanadium, Antipyrine and index of vanadium over Antipyrine expressed as Mean±SEM. T - test value:NS

absorption / elimination rate of V, antipyrine and index or V/Antipyrine with 1g/l glucose



Mean ± Sem, Statisitcal significance p>0.05, so NS

VANADIUM 2 G/L

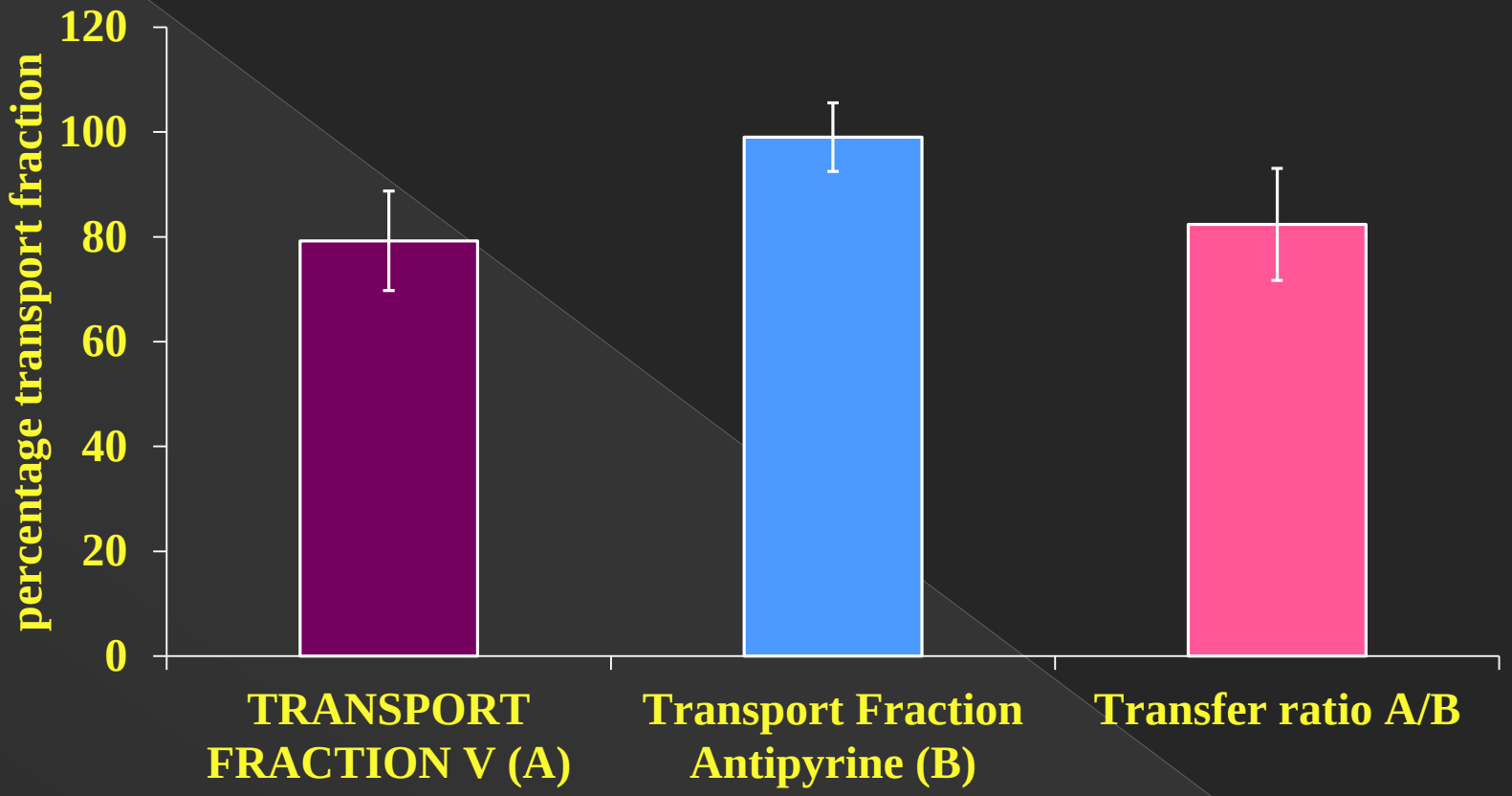
Differential transport rate for vanadium and Antipyrine in hyperglycemia

	10	25	50	75	90	Cotyledon wt
antipyrine	0.493 ± 0.04	1.22 ± 0.04	2.44 ± 0.05	3.66 ± 0.05	4.39 ± 0.06	31.67 ± 1.8
vanadium	0.57 ±0.053	1.300 ± 0.05	2.511 ± 0.06	3.72 ± 0.07	4.45 ± 0.08	31.67 ± 1.8
Significance	NS	NS	NS	NS	NS	

Pharmacokinetic parameters of Antipyrine and vanadium in hyperglycemia

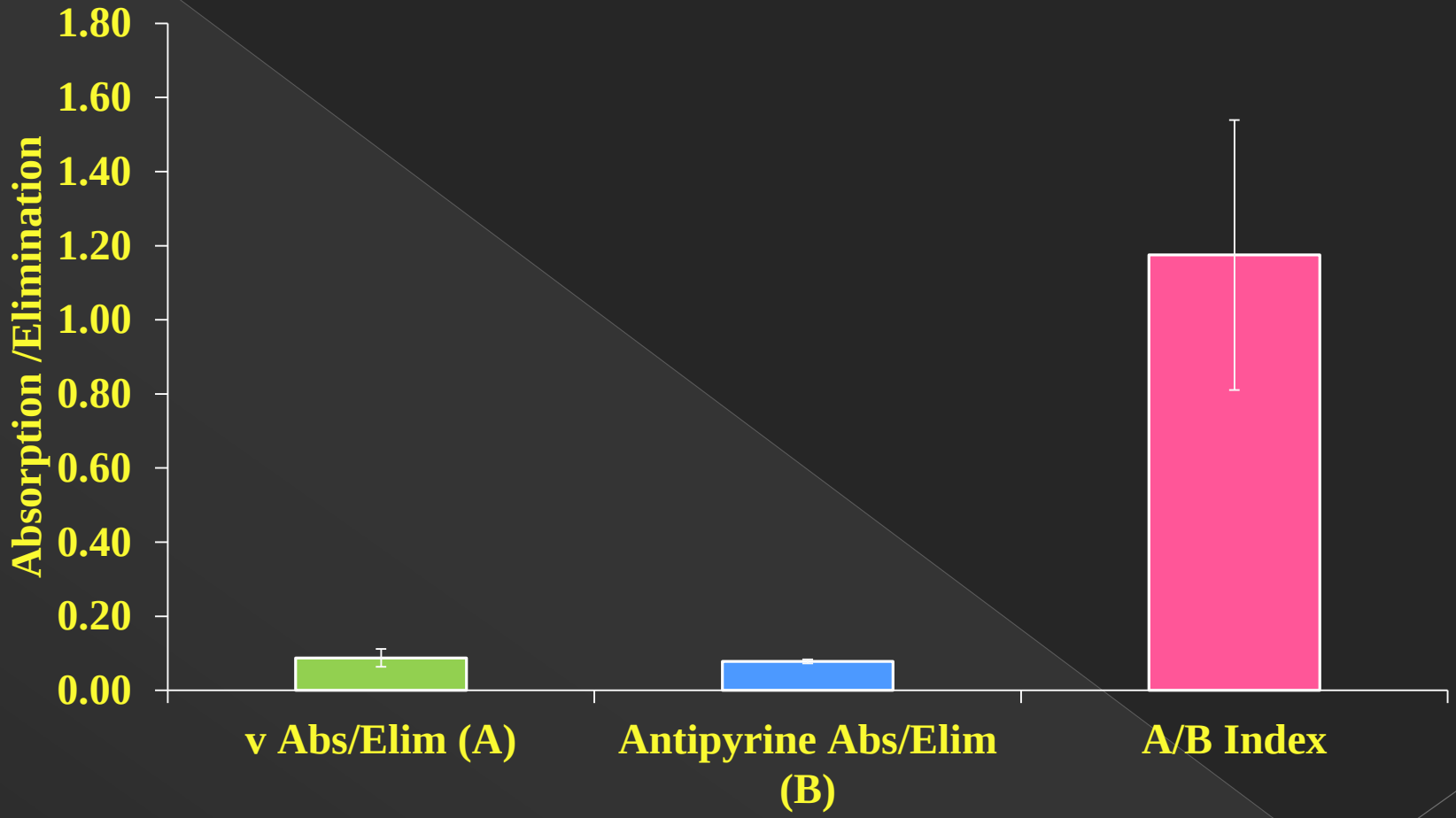
	AUC (ug-sec/l)	clearance (l/sec)*10^7	$kelsec^{-1}$	Tmax (sec)	absorption rate ug/sec *10^7	Elimination rate ug/sec
Antipyrine	2750.95 ± 620.3	0.0056 ± 0.0006	0.285 ± 0.024	150 ± 47.8	0.045± 0.013	0.581± 0.140
VANADIUM	11617.97 ± 1405.9	0.000011 ±0.000	0.277 ±0.006	150.0 ± 13.8	0.00029 ± 0.0002	0.0035 ± 0.0004
Significance	P<0.0001	P<0.0001	NS	NS	P<0.0001	P<0.0001

Transport fraction of vanadium and Antipyrine with 2g/l glucose



Transport fraction of vanadium, Antipyrine and index of vanadium over Antipyrine expressed as Mean±SEM. T - test value: NS

absorption / elimination rate of V, antipyrine and index or V/Antipyrine with 2g/l glucose

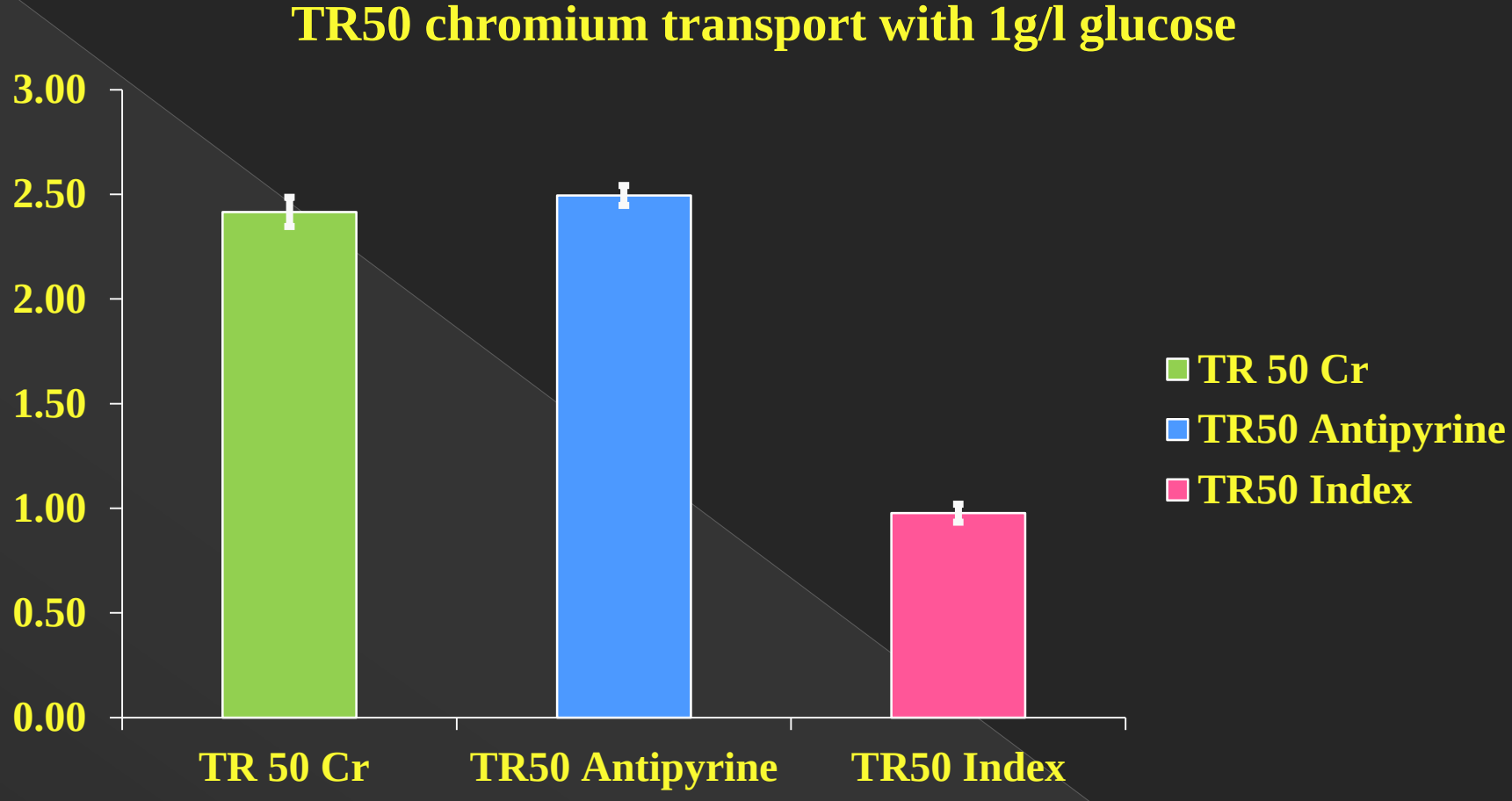


Mean ± Sem, Statisitcal significance p>0.05, so NS

CHROMIUM 1 G/L

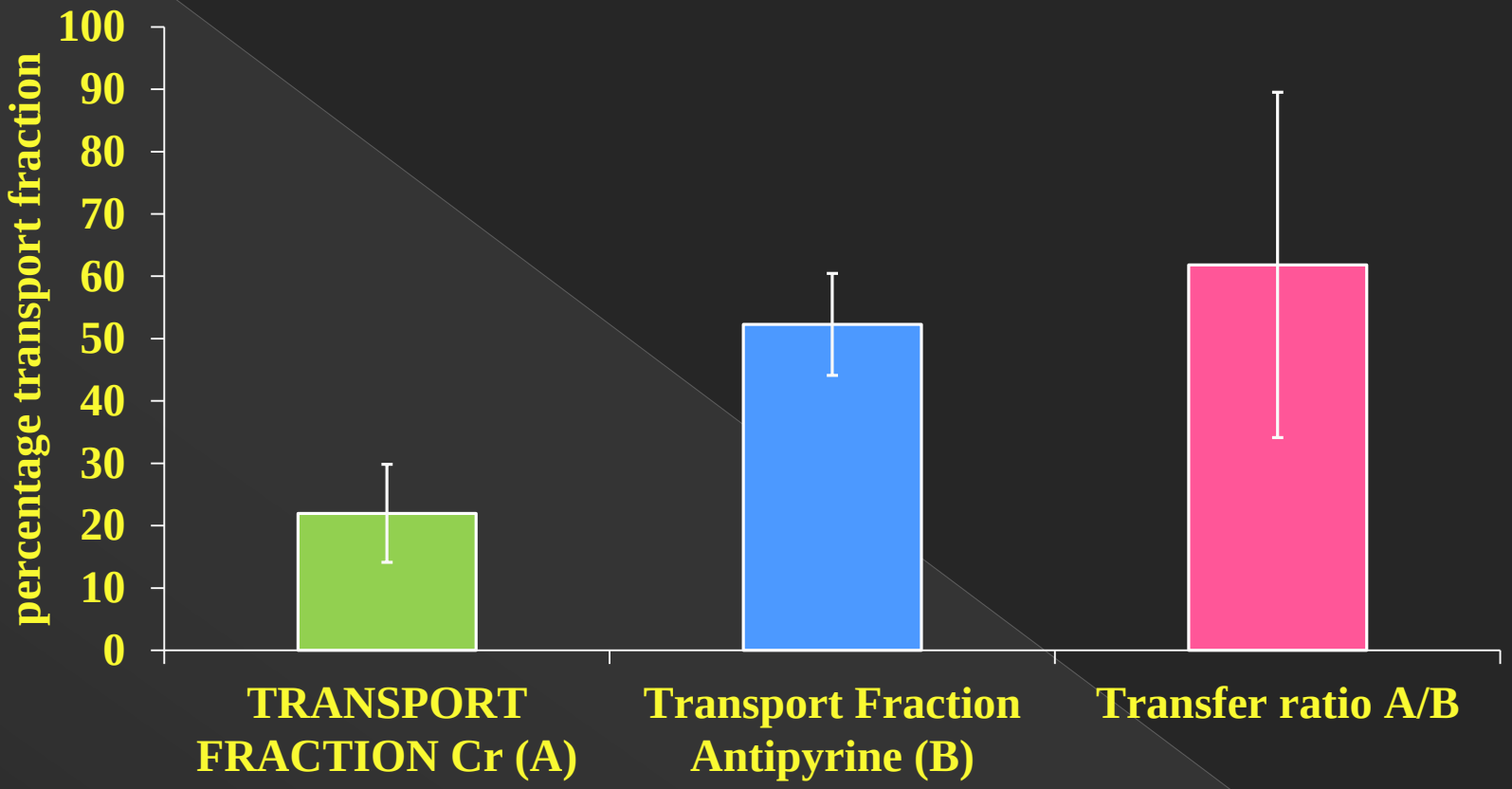
Differential transport rate of antipyrine and chromium in normoglycemia

	10	25	50	75	90	Cotyledon wt
antipyrine	0.611± 0.06	1.317 ± 0.05	2.494± 0.04	3.672 ± 0.03	4.37 ± 0.03	31.67 ± 1.8
chromium	0.486 ± 0.05	1.20 ± 0.06	2.41 ± 0.07	3.62 ± 0.08	4.34 ± 0.09	31.67 ± 1.8
Significance	NS	NS	NS	NS	NS	



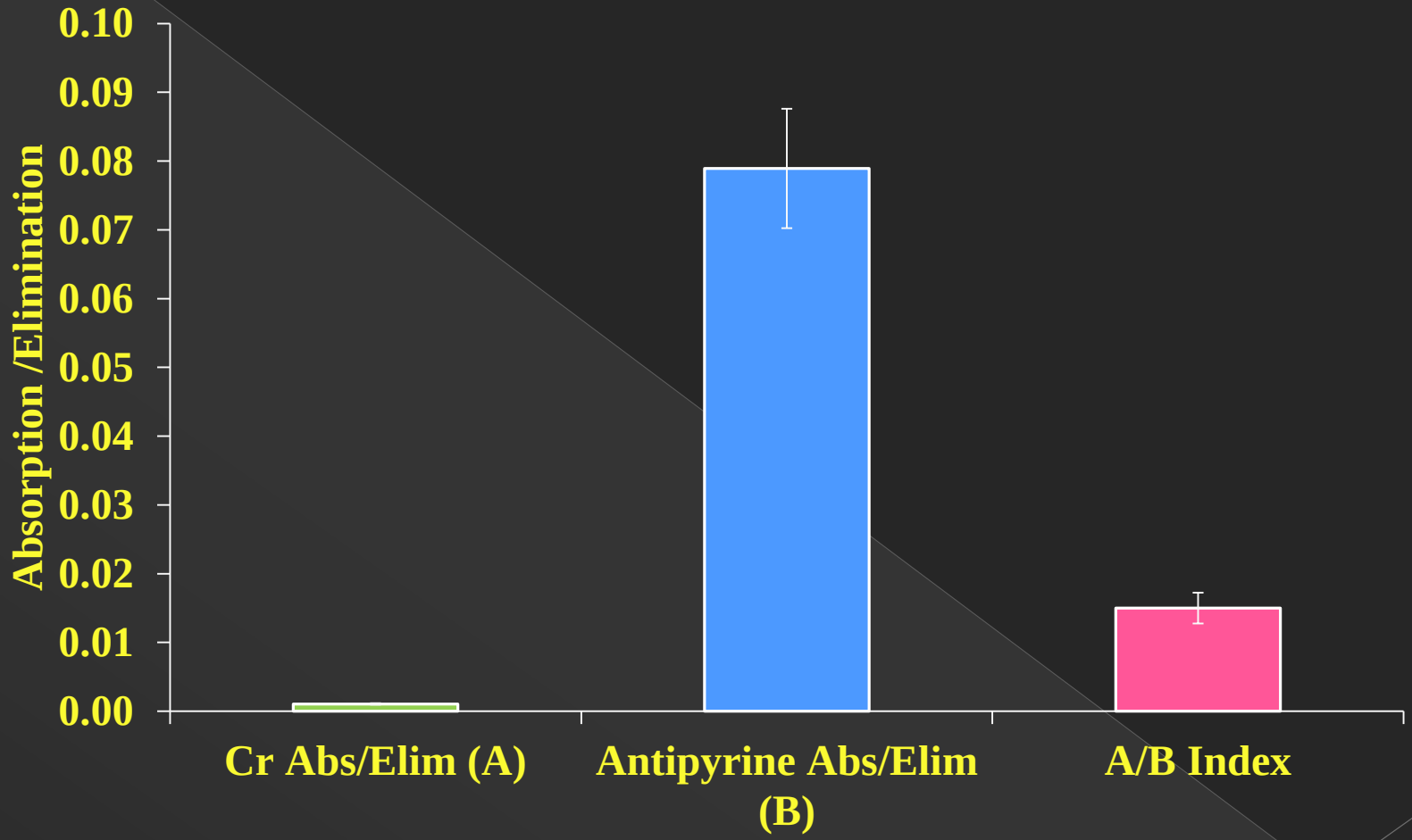
TR 50 of chromium, Antipyrine and index of chromium over Antipyrine expressed as Mean±SEM.

Transport fraction of chromium and Antipyrine with 1 g/l glucose



Transport fraction of chromium, Antipyrine and index of chromium over Antipyrine expressed as Mean±SEM. T - test Significance p=0.013

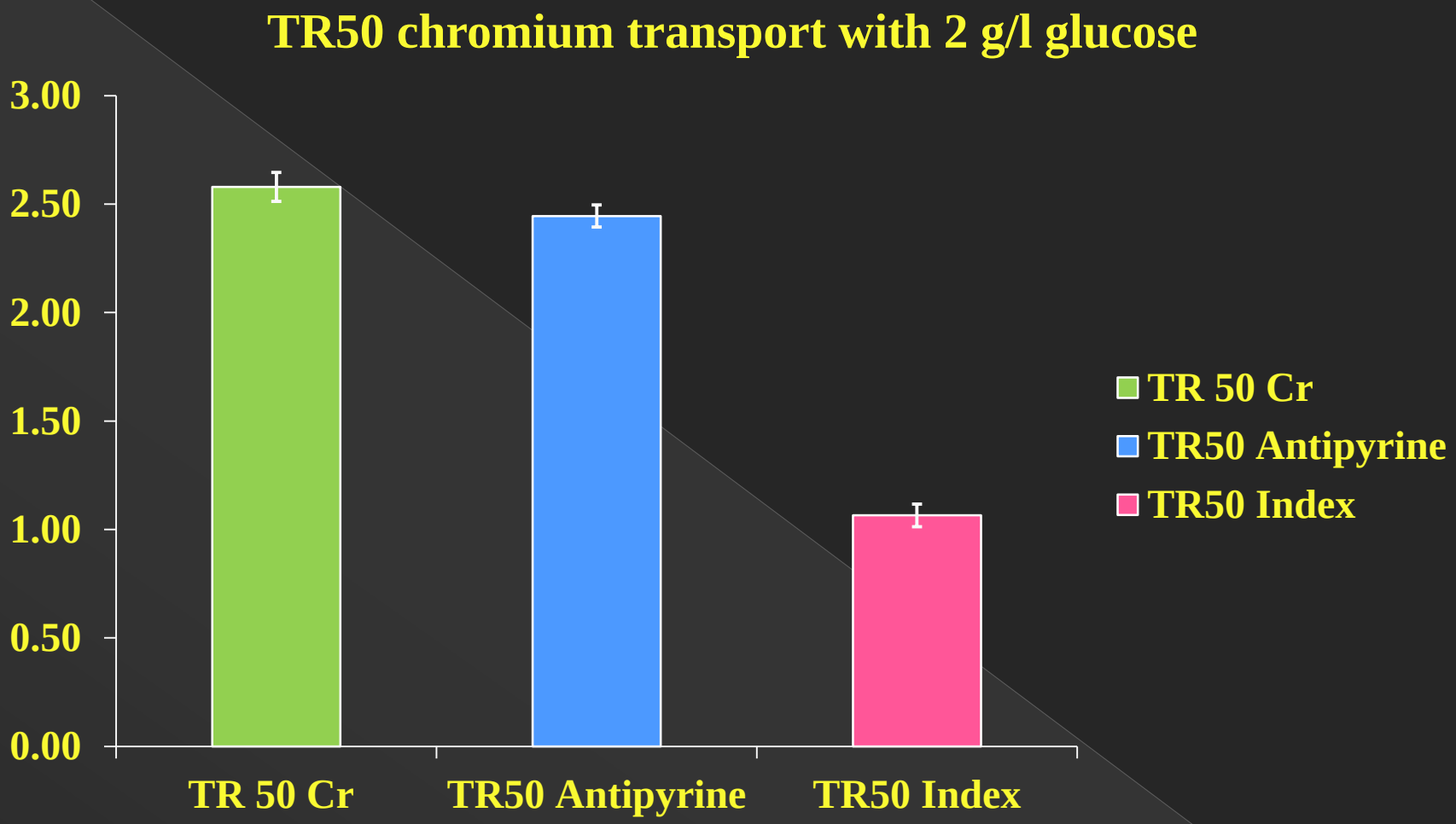
Absorption / Elimination rate of Cr , antipyrine and index or Cr/Antipyrine with 1g/l glucose



Mean ± Sem, Statisitcal significance $p<0.001$, so statistically significant.

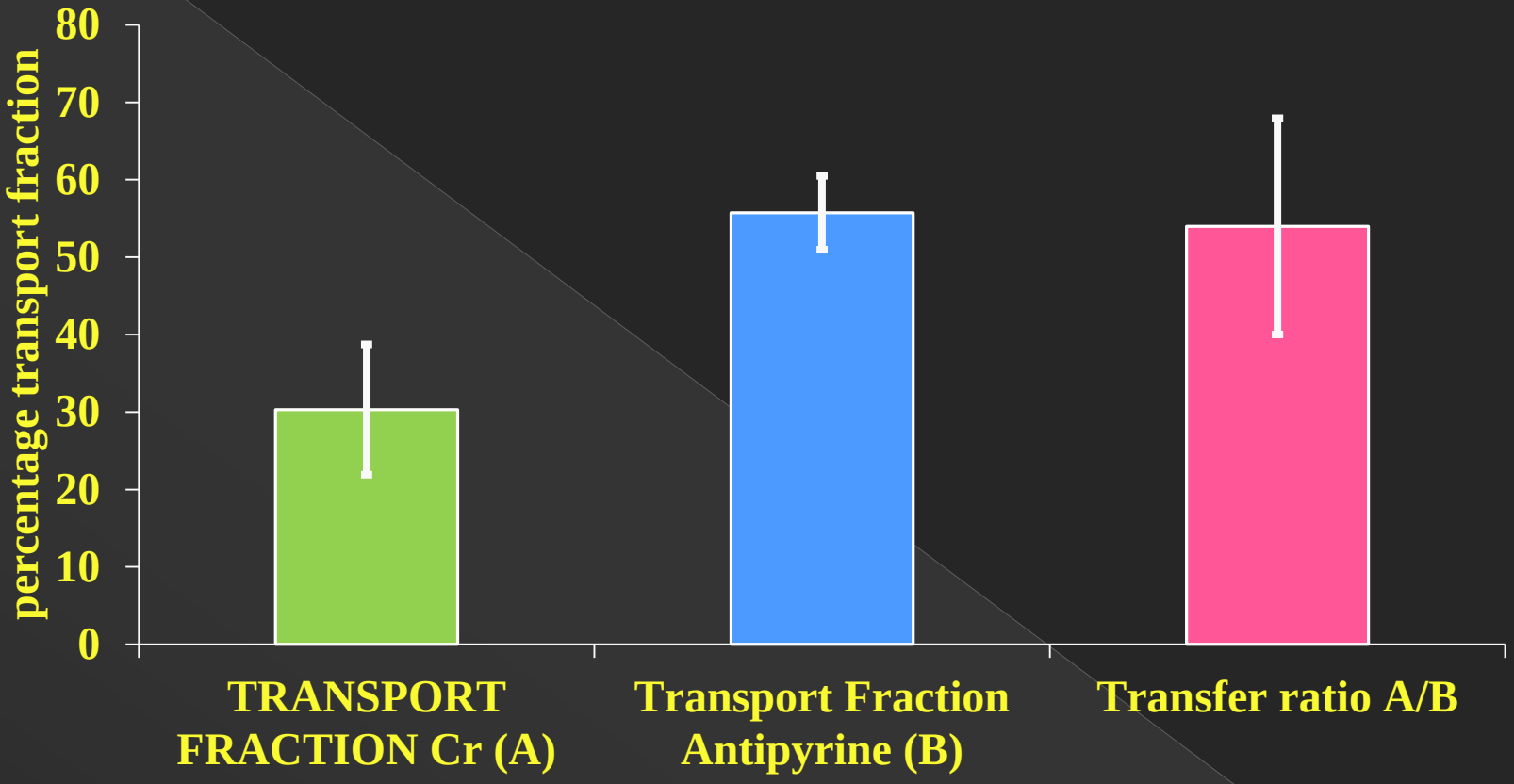
Pharmacokinetic parameters of Antipyrine and chromium in hyperglycemia

	AUC (ug-sec/l)	clearance (l/sec)*10^7	Kel sec^{-1}	Tmax (sec)	absorption rate ug/sec *10^7	Elimination rate ug/sec
antipyrine	2750.95 ± 620.3	0.0056 ± 0.0006	0.285 ± 0.024	150 ± 47.8	0.045± 0.013	0.581± 0.140
chromium	286.76 ± 258.94	5.41 ± 0.46	0.27 ± 0.023	150 47.8634 4	6.91 ± 6.75	0.0006 ± 0.0007
Significance	P<0.0001	P<0.0001	NS	NS	P=0.00 19	P<0.0001



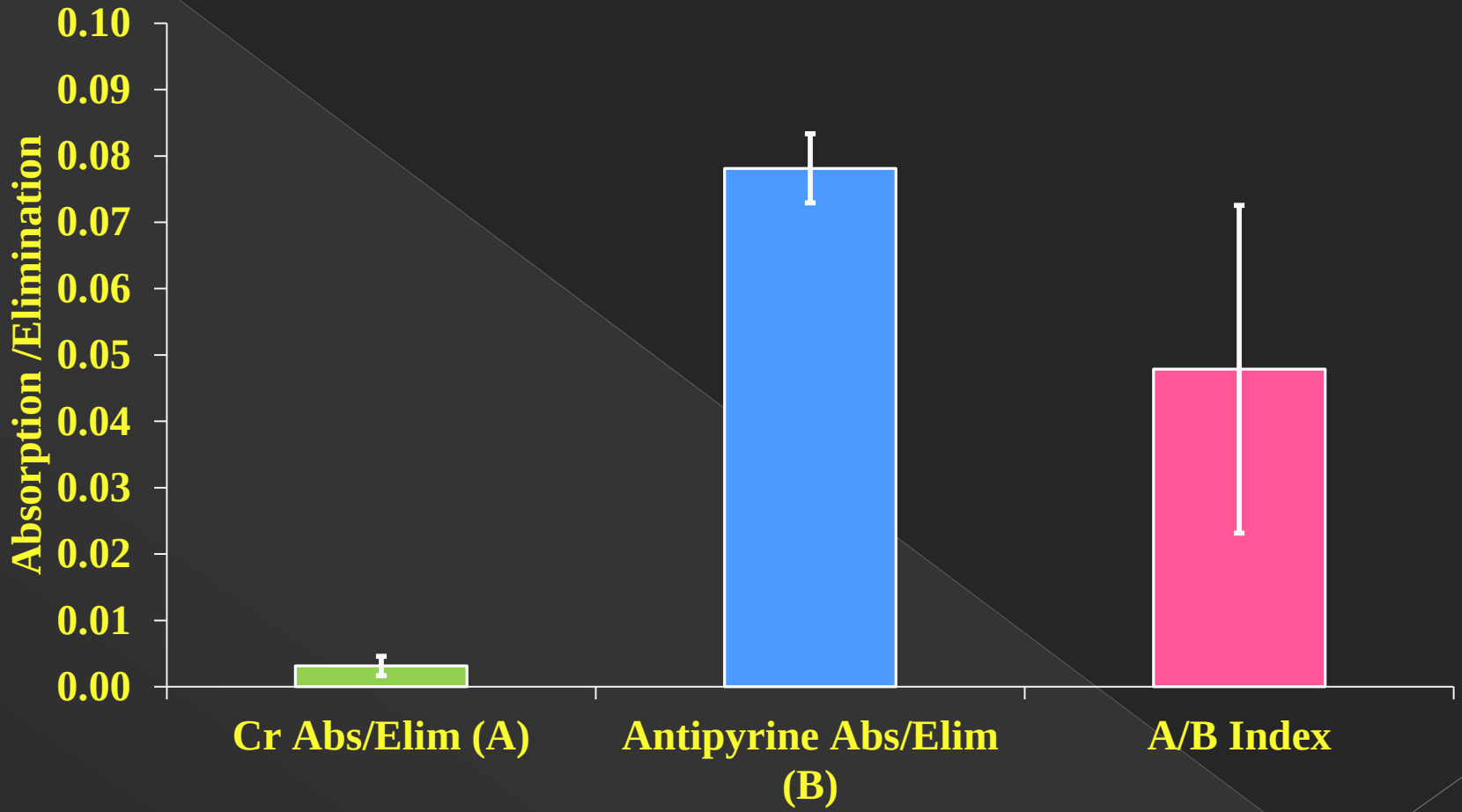
TR 50 of chromium, Antipyrine and index of chromium over Antipyrine expressed as Mean±SEM.

Transport fraction of chromium and Antipyrine with 2g/l glucose



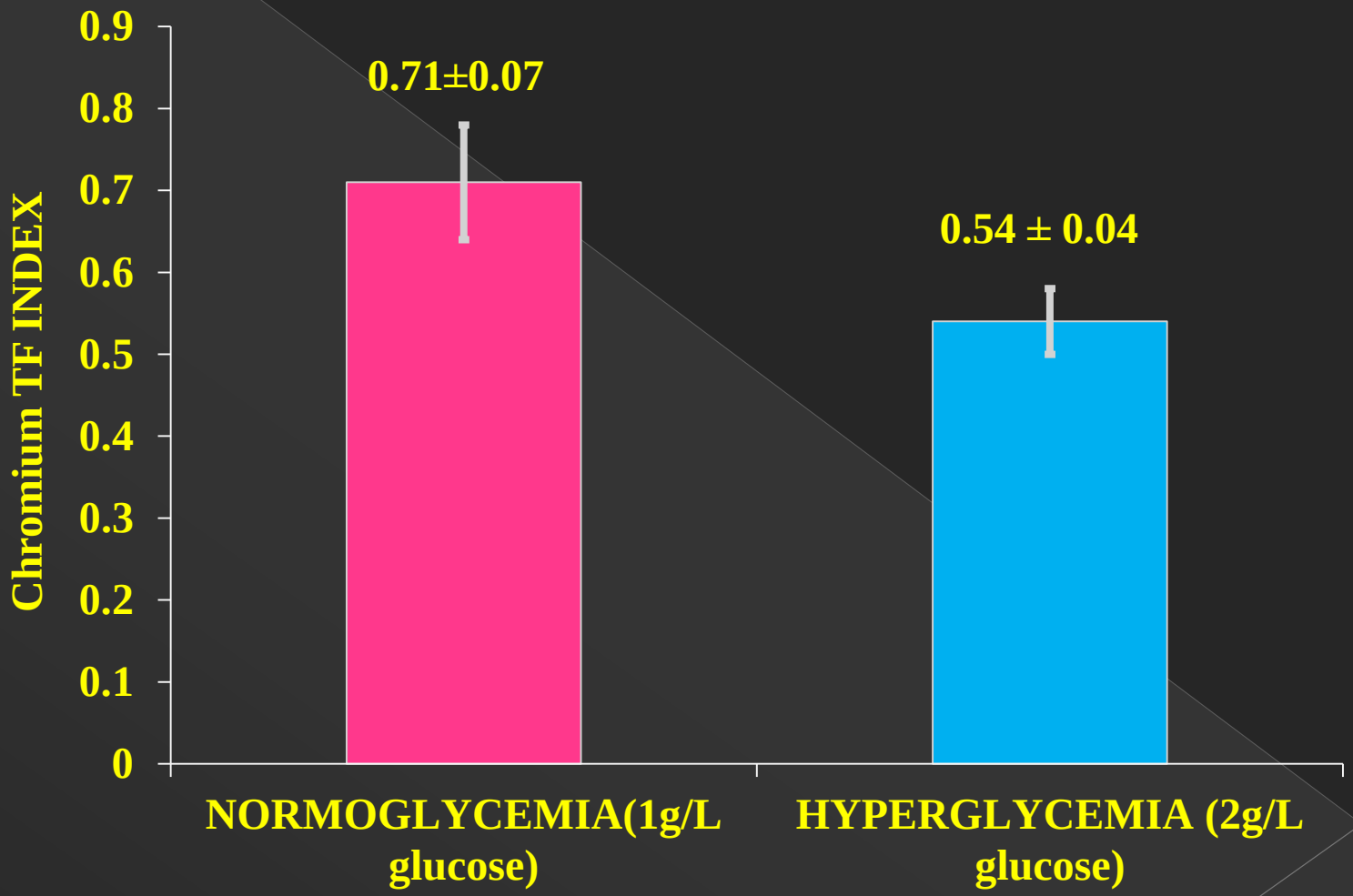
Transport fraction of chromium, Antipyrine and index of chromium over Antipyrine expressed as Mean±SEM. T - test Significance p=0.017

absorption / elimination rate of Cr , antipyrine and index or Cr/Antipyrine with 2g/l glucose

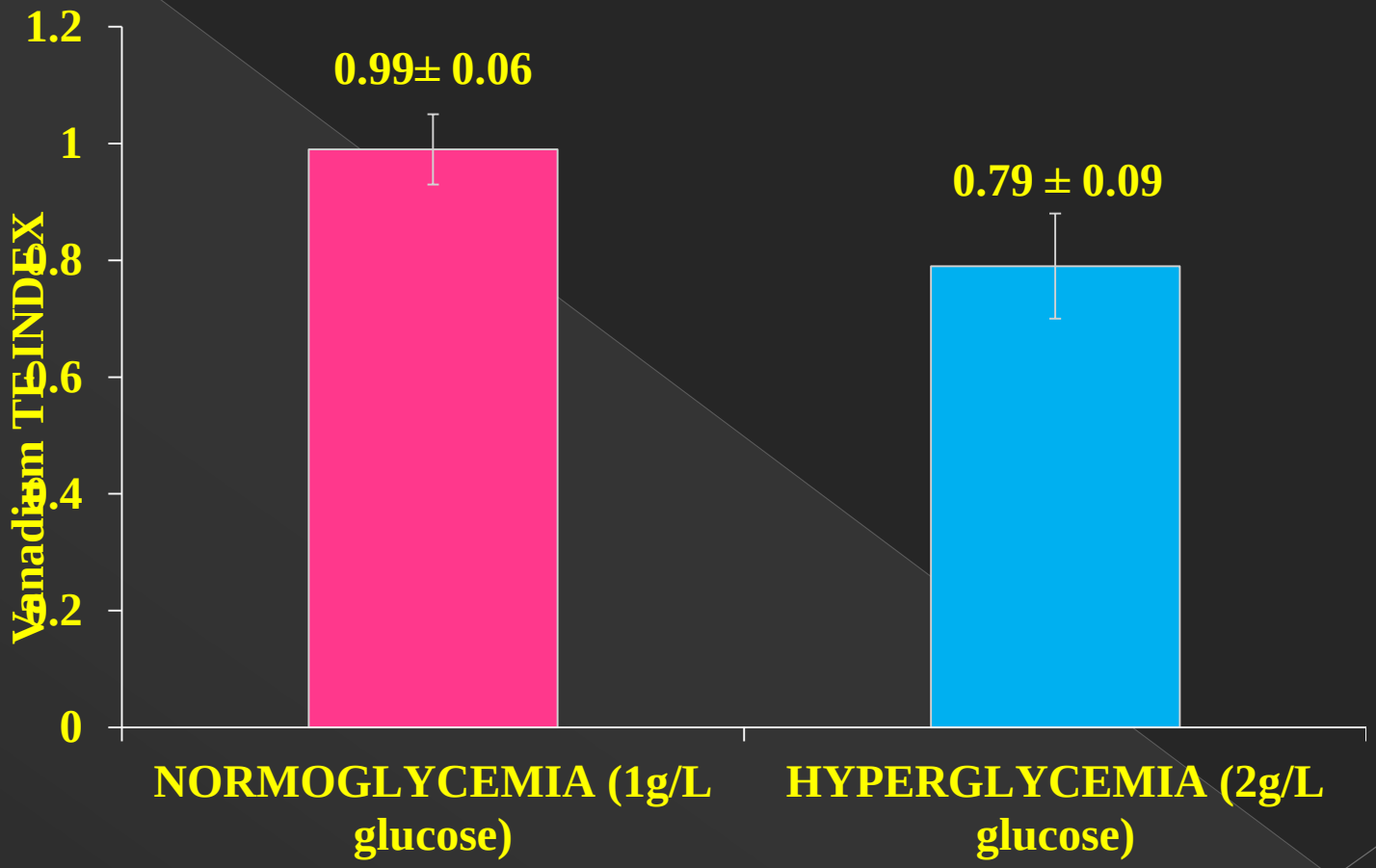


Mean ± Sem, Statisitcal significance p<0.001, so statistically significant

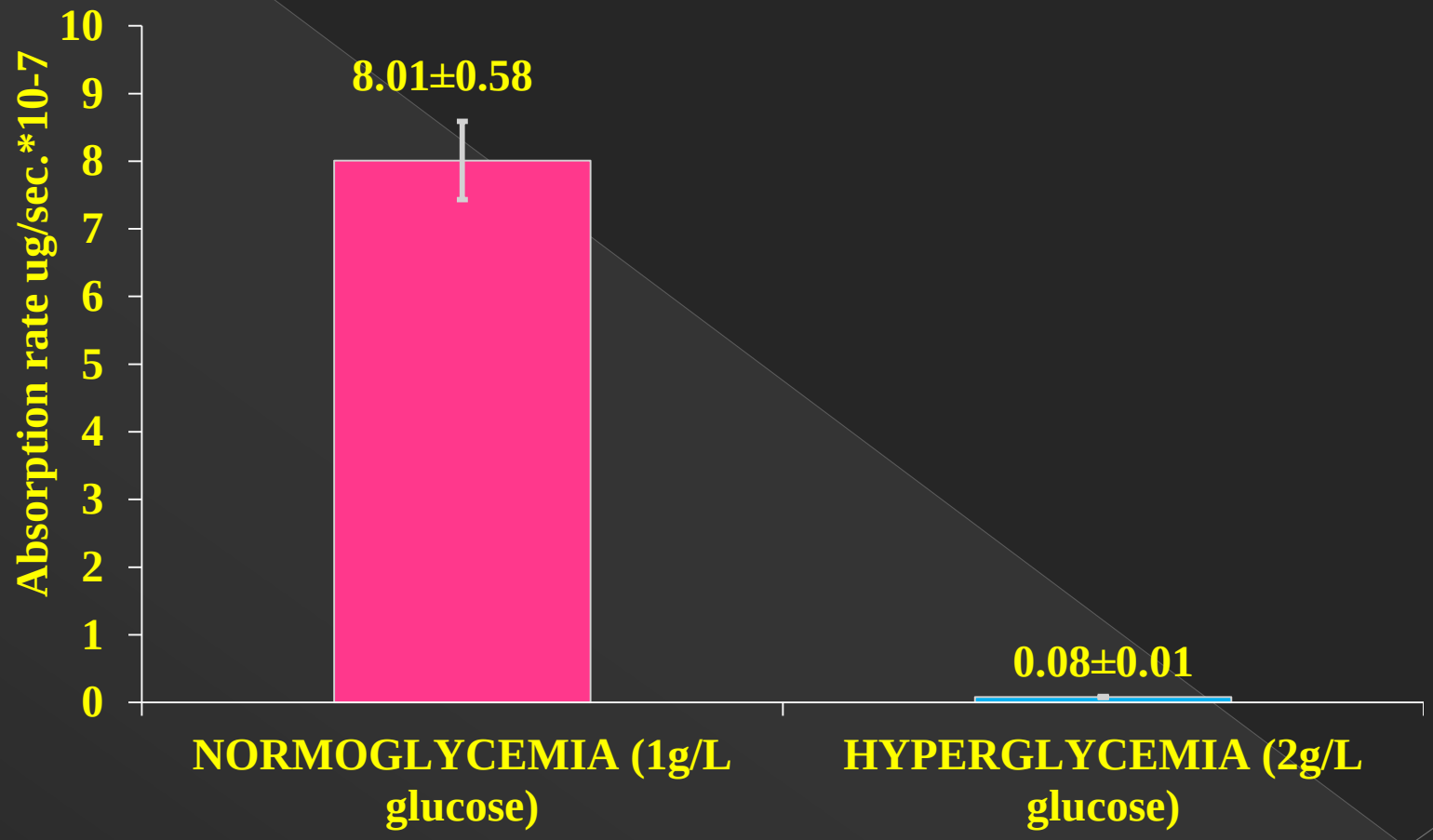
CHROMIUM PERFUSIONS



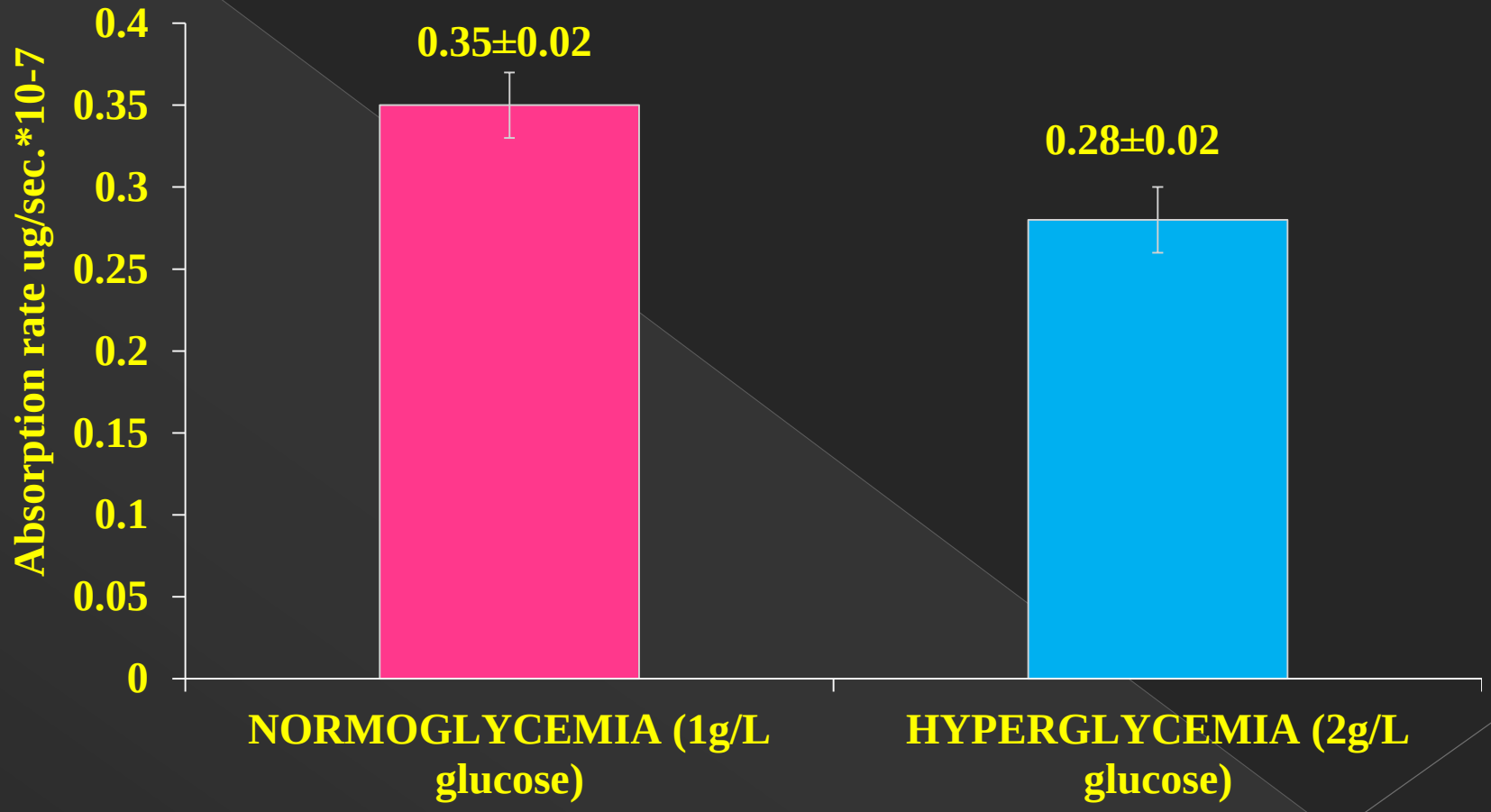
VANADIUM PERFUSIONS



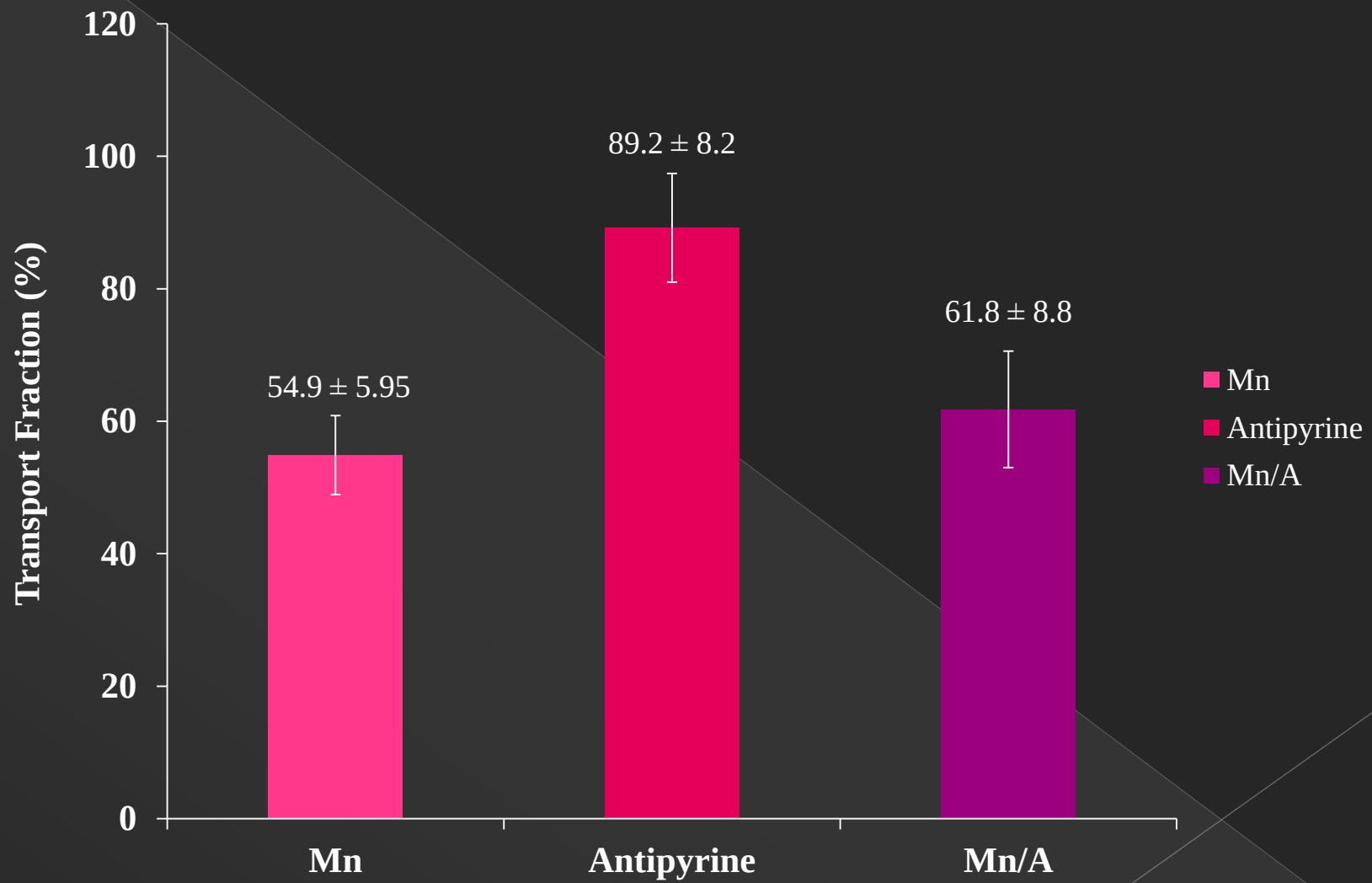
CHROMIUM PERFUSIONS



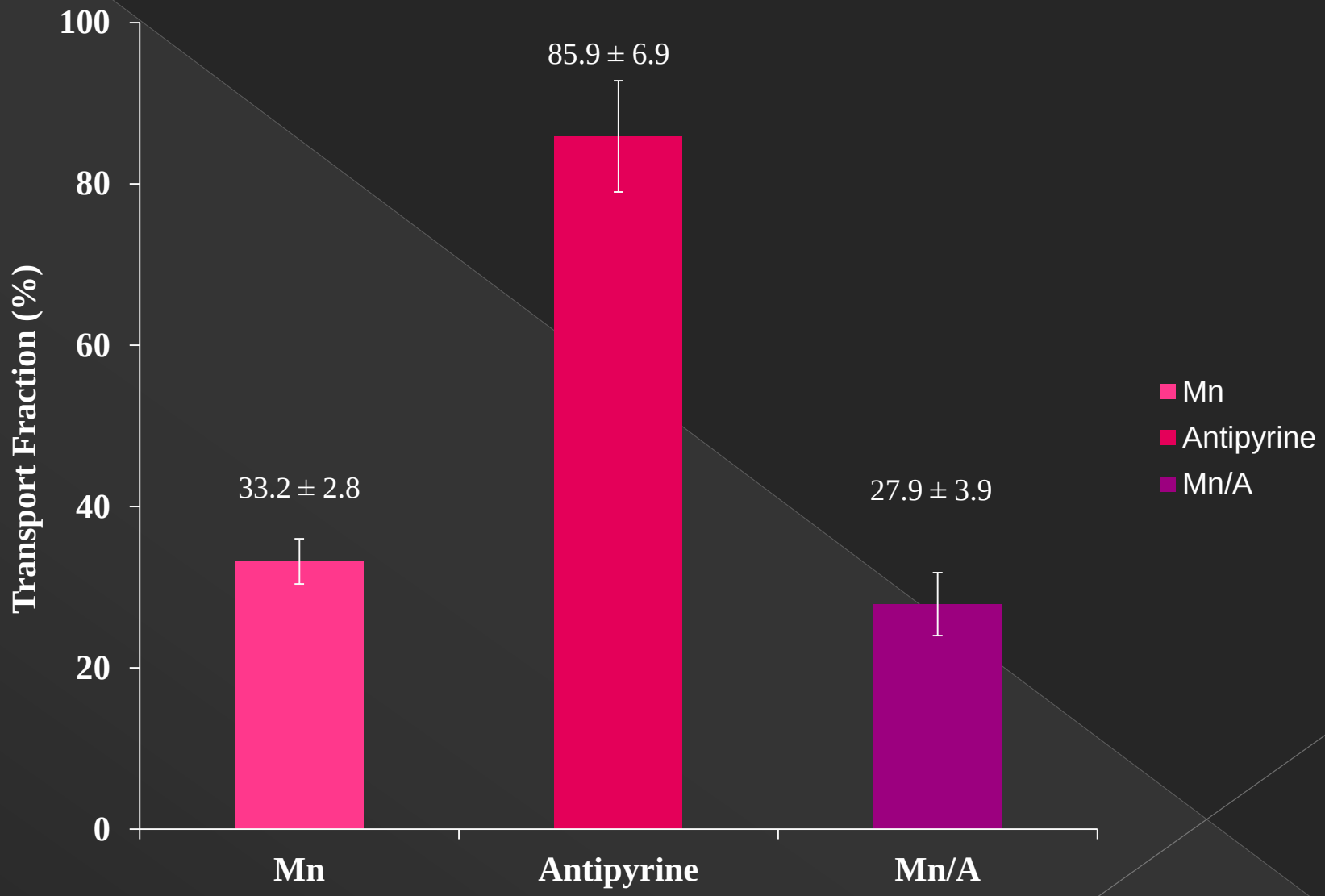
VANADIUM PERFUSIONS



Normoglycemic Perfusions (1 g/L)



Hyperglycemic Perfusions (2 g/L)



DISCUSSIONS

Previous research from our laboratory had demonstrated maternal -fetal transport kinetics of selenium and zinc were compromised in placentae of gestational diabetic patients compared to control pregnant women as well as in experimentally induced diabetic pregnant rats ***(Nandakumaran et al, 2006, Al-saleh, Nandakumaran et al, 2004, Al-saleh, Nandakumaran et al, 2005)***

Our Data show for the First Time detailed transport kinetics of relatively newly recognized essential trace elements in the human placenta in vitro

Chromium transport from maternal to fetal circulation is relatively lower compared to vanadium and this was surprising since both are low molecular weight elements and hence the possibility of a carrier mediated transport cannot be ruled out in their transport across the human placenta in vivo as well

The data on antipyrine or reference marker transport are consistent with the free permeability of the marker reported by us in the human placenta in vitro ***(Nandakumaran et al, 1981, 2004)*** **as well as with those of other international research groups** ***(Meschia et al, 1967, Challier et al, 1986).***

We further speculate that vanadium is actively transported from mother to fetus and this is corroborated by the lower T50 values in all our perfusion conditions compared to antipyrine and the situation is likely to be compromised in hyperglycemic states as well

Moderate hyperglycemia of 11 mmol/L was shown to decrease transport of chromium, manganese as well as vanadium compared to euglycemic state in our perfusion conditions.

- We report for the First Time in literature maternal-fetal transport of Mn from maternal to fetal circulation in human placenta in vitro.Considering the Restricted transport of this element despite its small atomic weight, we believe that like Cr&V, Mn too is actively transported from mother to fetus in humans.Further studies are on

Both the transport fractions and areas under the curves and absorption rates of above elements were significantly lowered in hyperglycemic states

Predictably antipyrine or reference marker transport was not affected by the hyperglycemic state .

Experiments are underway to establish whether higher glucose load as in uncontrolled diabetes of say 27mmol/L of glucose can lower the maternal fetal transport of above elements.

This further could seriously impair the fetus or neonatal metabolism and carbohydrate metabolism in particular in those states.

The above possibility has been established in 2 perfusions from highly diabetic patients and more studies are underway to ascertain the veracity and statistical significance of above findings

We recommend that chromium and vanadium and manganese levels of pregnant women need to be monitored carefully particularly in pregnant diabetic patients and those liable to have gestational diabetes.

Control of blood sugar in pregnancy deserves to be done more aggressively to prevent compromised transport of essential trace elements such as vanadium and chromium as well

Further studies are underway to evaluate the possible effect of binding of above trace elements to placental tissue proteins in control and diabetic pregnancies.

Furthermore assessment of the probable effect or relationship of vanadium and chromium levels to the antioxidant enzyme activity in control and diabetic pregnancies are to be studied

Acknowledgement

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Thank You
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Water towers

Green island

The Kuwait Towers

Liberation tower

Al-Kout Mall

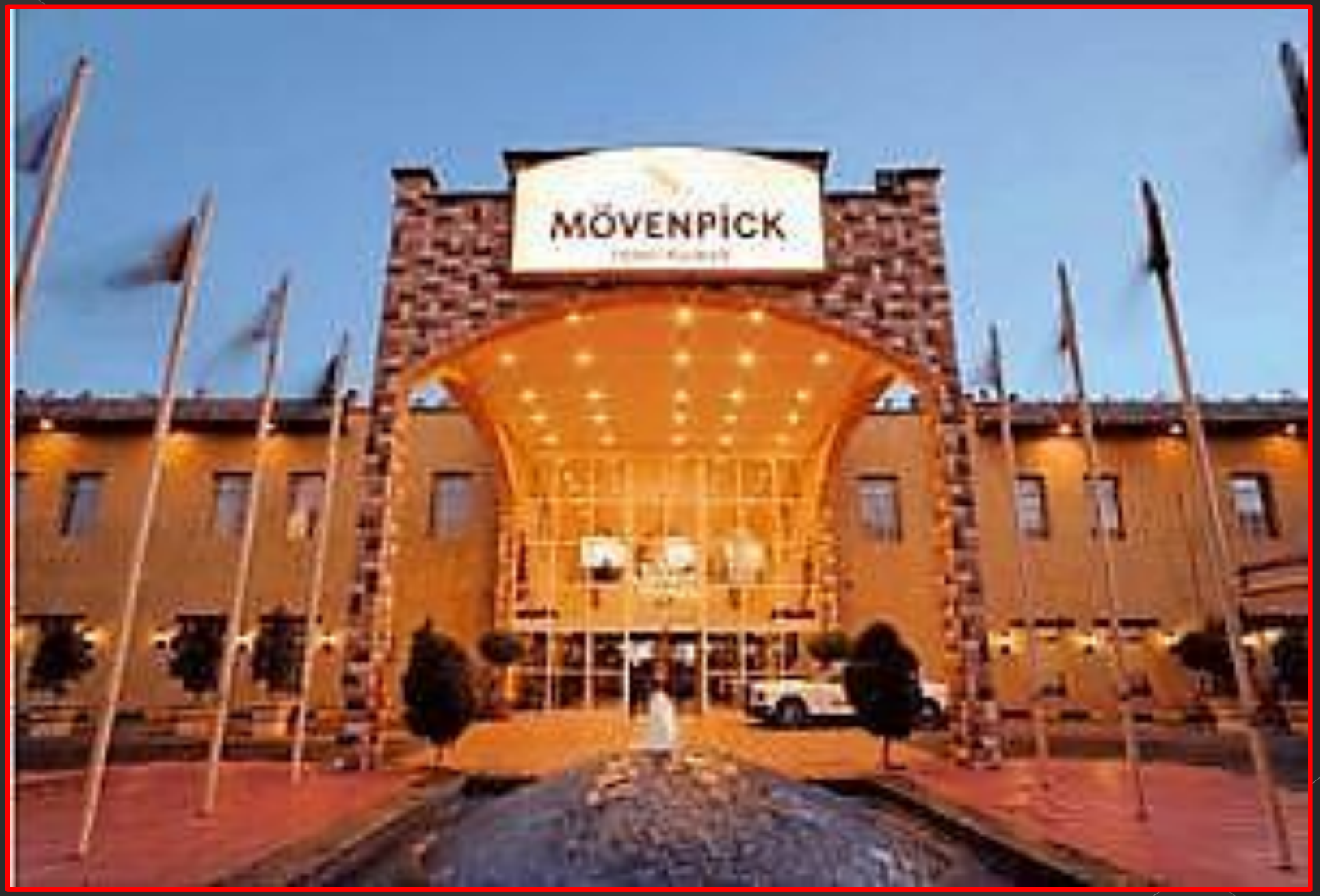
Date palm

Grand Mosque Kuwait

The Kuwait Airport


the palms
النخيل
The Palms Beach Hotel & Spa


Sheraton
شيراتون


MÖVENPICK

360 mall

Avenues mall

Oil plant