

To investigate effects of a shifting high fat diet to normal fat diet supplemented with magnesium, zinc and chromium on biochemical parameters in rats with diabetes

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Several minerals play an important role in modulating blood glucose and thyroid hormones. The aim of this study was to investigate the effect of individual and combined minerals; chromium (Cr), magnesium (Mg) and zinc (Zn) on blood glucose, lipid profile, kidney and liver functions, T_3, T_4 and TSH among obese diabetes rats. The study was carried out on 66 male Wistar rats (150-160g) for 90 days on two stages. First stage included 66 rats (6 of which were control) which were fed on a high fat diet (19% hydrogenated fats and 1% corn oil as a source of essential fatty acids) for 45 days. Second stage of the study included the same 66 rats (6 of which were control) which were injected with (150 mg Alloxan / kg b.w) to induce experimental diabetes and were then fed normal levels of dietary fat supplemented with individual and combined minerals; Cr, Mg and Zn at two levels (high and low). Results indicate that in the positive control rat group (PC) there was elevated cholesterol and triacylglycerol levels. While, rat groups supplemented with combined elements (at low and high levels) led to lowering cholesterol and triacylglycerol significantly ($p < 0.05$). In particular, Cr had improved triacylglycerol status. The vice versa was noticed in rat groups fed on diets supplemented with individual trace elements. HDL lipoprotein levels were increased in individual supplementation with zinc (20mg) and at higher levels of Mg, Zn and Cr. Cr, at higher levels (200 µg) led to significant reduction of the VLDL-C compared with negative control (NC) group. Uric acid, urea nitrogen and creatinine were decreased significantly with diets supplemented with individual Zn at higher levels (20mg/ kg b.w). The combination at high levels showed reduction of uric acid, urea nitrogen and creatinine compared to NC. Conclusion: our results indicate that diets supplemented with combined elements led to improving the tested parameters in this study.

Key words: diabetes mellitus, rats, alloxan, lipid profile, liver function, thyroid hormones

INTRODUCTION

Obesity and the incidence of type II diabetes are increasing worldwide. The development of complications in diabetes are well reviewed, with an associated increase in morbidity and mortality.

Minerals have been shown to influence hormones at several levels, including hormone secretion and activity, and binding to the target tissue. Moreover, hormones have been shown to influence trace metal metabolism at several levels, including excretion and transport of trace metals (Henkin 1976). Magnesium is a macro mineral which is known to play an important role in carbohydrate metabolism, and its imbalance has been implicated in diabetes mellitus both as a cause and a consequence (American Diabetic Association1992; Mooradian and Morley 1987).

Magnesium deficiency has been associated with type II diabetes and may reduce insulin sensitivity and impair glucose tolerance. The aetiology of magnesium depletion in diabetes is unclear. Animal studies suggest that diabetes may impair magnesium absorption; however, there are no published data on magnesium absorption in humans with diabetes (Wälti et al. 2003).

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Chromium (Cr) is one of the few trace minerals for which a specific cellular mechanism of action has not been identified. Recent *in vitro* studies suggest that Cr supplementation may improve insulin sensitivity by enhancing insulin receptor signalling, but this has not been demonstrated *in vivo*. In a study, investigating the effect of Cr supplementation on insulin receptor signalling in an insulin-resistant rat model, the JCR:LA-corpulent rat, the data suggested that chromium supplementation of obese, insulin-resistant rats may improve insulin action by enhancing intracellular signalling. (Wang et al. 2006).

In addition to humans, beneficial effects of supplemented Cr have been observed in rats, mice, squirrel, monkeys, guinea pigs, rabbits, fish, pigs, cattle and horses (Anderson 1993; Mertz 1993). In a cohort of type II diabetes patients, low serum zinc (Zn) level was an independent risk factor for CHD events (Soinio et al. 2007).

While it is clear that urinary excretion of Zn is markedly increased in individuals with diabetes, if hyperglycaemia is the primary aetiology, replacement with oral Zn supplementation should provide sufficient treatment. High dose supplementation in Type I diabetes and in normal individuals was evaluated by serum and urine Zn and mononuclear cell Zn concentrations. Zn excretion and mononuclear Zn concentrations were increased by a

similar amount in both groups. There did not appear to be any evidence for total body Zn deficiency in the group with diabetes despite the clear hyperzincuria. Somewhat distressing in this study was an increase in haemoglobin Alc in all individuals which was, of course, of greater concern in the subjects with diabetes. The data suggested that there is a possible Zn toxicity for high dose supplementation which has greater ramifications in subjects with diabetes. It also suggests that there is more than hyperzincuria responsible for the abnormalities in Zn metabolism in patients with diabetes (Cunningham et al.1994).

The aim of this study was to explore the effects of diets supplemented with certain individual and combined elements such as chromium (Cr), magnesium (Mg), and zinc (Zn), on blood sugar level, lipid profile, liver and kidney functions, T_3, T_4 and TSH among male Wistar rats. These minerals were studied extensively but the combinations of these minerals were lacking in the literature. Furthermore, in this study the amounts of minerals used in the supplemented diets were different compared to similar studies. The model followed in this study is matching the scenario of obesity that is associated with several complications such as type II diabetes (Environmental Diabetes).

MATERIALS AND METHODS

Diets and chemicals

All diet components such as casein, vitamins, minerals, fibre, were obtained from El-Gomhoreya Company, Cairo, Egypt. Kits used to carry out biochemical parameters in this study were obtained from Egyptian American Company for Laboratory Service.

Experimental Animals

This study was performed on (n = 66) male Wistar rats at 8 weeks of age, the rats were housed and bred as approved by the Animal Ethics of Nutrition and Food Science Department; Faculty of Home Economics, Helwan University, Egypt. Rats were kept separately in metal cages in a room with controlled temperature (20 to 22°C) and humidity (50 to 55%), and maintained in a cycle of light for 12 h (06:00 to 18:00 h) and dark for 12 h (18:00 to 06:00 h). Rats were allowed to consume their respective diets (Table 2) and water as *ad libitum*. The animals were kept on rodent chow for a week. After this washout period, rats were divided into 2 groups; group A (n=60) (150-160g) fed on high fat diet (19% hydrogenated fats and 1% corn oil as a source of essential fatty acids) and group B (6 rats) fed on basal diet Ain-93M (Reeves et al. 1993) for 45 days. Using hydrogenated fats in this experiment aimed to induce experimental obesity and increase the risk factor to heart disease as it's a source of trans fatty acids. After this stage, rats (n=66) were divided into 11 sub-groups as explained in Table 1. Sixty of which (n=60) were injected with Alloxan to induce diabetes, (apart from control group n=6), within 48 hours from injection, blood sugar was elevated to above 200 mg/dl. In this stage, rats were (n=60) were fed on normal level of fat according to Ain-93M. Diets were supplemented with either individual or combined minerals; Cr, Mg, and Zn at two levels as presented in Table 1. These supplements were given by tube feeding daily. Lipid profile was measured during the study by taking blood samples from the orbital plexus of all rats, in order to confirm the elevation of lipid profile parameters (Data not shown) compared to control group.

Rats were sacrificed after 45 days of feeding on diets supplemented with individual and combined minerals. Body weight was recorded, blood collected from orbital plexus for biochemical analyses: a series of biochemical analyses were measured; cholesterol, triacylglycerol, LDL-C, VLDL-C blood glucose, uric acid, urea nitrogen, creatinine, AST, ALT, T3, T4, and TSH.

Table 1: Schematic of experimental rat groups

Negative control group (NC)	
Obese diabetes group (PC)	
Obese diabetes rat groups fed on normal dietary fat and treated daily by oral dose of:	Distilled water group (DW)
	200-mg magnesium sulphate / (kg bw) (Mg 200)
	300 -mg magnesium sulphate / (kg bw) (Mg 300)
	10 mg zinc chloride / (kg bw) (Zn 10)
	20 mg zinc chloride / (kg bw) (Zn 20)
	100 µg chromium / (kg bw) (Cr 100)
	200 µg chromium / (kg bw) (Cr 200)
	200-mg magnesium sulphate, 10 mg zinc chloride and 100 µg chromium / (kg bw) (Combined low level)
	300-mg magnesium sulphate, 20 mg zinc chloride and 200 µg chromium / (kg bw) (Combined high levels)

Table 2: Composition of the basal diets

Ingredient g/kg Diet	Control	High fat
Casein	140.000	140.000
Fibre	50.000	50.000
*Corn oil/hydrogenated fats	40.000	200.000
Mineral mix.	35.000	35.000
Vitamin mix.	10.000	10.000
Corn starch	465.692	345.692
Dextrinized corn starch	155.000	155.000
Sucrose	100.000	100.000
L-cytesine	1.800	1.800
Cholin bitartarate	2.500	2.500
Tert-butylhydroquinone	0.008	0.008

*Soybean oil was replaced by corn oil

Blood glucose was measured by (Trinder 1959), total thyroxin T_4 (Britton et al. 1975), triiodothyronine T_3 (Ahmed et al. 1974) and thyroid-stimulating hormones TSH (Wada et al. 1983).

Table 3: Effect of diets supplemented with Cr, Mg and Zn on weights of obese rats with diabetes.

Groups	Parameters	Weights			BWG%
		Initial	Middle	Final	
Negative control group (NC)		230.8±3.7^{b}	256.6± 5.2^{e}	274.5± 7.6^{h}	18.9± 4.3^{fg}
Obese diabetes group (PC)		281.3± 5.9^{a}	331.0± 4.0^{a}	386.6± 8.4^{a}	37.4± 2.3^{a}
Obese diabetes rat groups fed on normal diet and treated daily by daily oral doses of:	Distilled water group (DW)	281.0±9.9^{a}	320.5 ± 4.3^{ab}	368.2±10.8^{b}	31.1± 1.6^{b}
	200 -mg magnesium sulphate / (kg bw)	280.8±9.0^{a}	306.5± 5.7^{abcd}	358.5± 5.7^{c}	27.8± 3.5^{c}
	300 -mg magnesium sulphate / (kg bw)	280.8± 9.1^{a}	296.0 ± 6.2^{bcd}	340.2± 5.9^{e}	21.2± 2.4^{ef}
	10 mg zinc chloride / (kg bw)	279.2±6.6^{a}	310.5 ±4.2^{abc}	362.5± 6.7^{bc}	29.8 ± 1.6^{bc}
	20 mg zinc chloride / (kg bw)	280.8 ±9.0^{a}	317.6 ± 37.9^{ab}	344.5± 8.4^{de}	22.7± 1.6^{de}
	100 µg chromium /(kg bw)	280.0± 3.7^{a}	305.2± 4.2^{abcd}	349.7 ± 5.6^{d}	24.9± 2.0^{d}
	200 µg chromium /(kg bw)	279.3±5.2 ^{a}	300.0±39.7 ^{bcd}	329.0± 5.8^{f}	17.8± 2.2^{g}
	200-mg magnesium sulphate, 10 mg zinc chloride and 100 µg chromium / (kg bw)	279.2 ± 2^{a}	285.8± 4.8^{cd}	329.7 ± 5.6^{f}	18.1±2.3^{g}
	300-mg magnesium sulphate, 20 mg zinc chloride and 200 µg chromium / (kg bw)	278.7± 5.1^{a}	282.0± 38.5^{d}	309.3 ± 6.3^{g}	11.0± 1.8^{h}

Significant differences at P < 0.05; NC= Negative control, (PC) Obese rats with Diabetes Group (High fat diet); Similar letters in the same column indicate non-significant differences; BWG% = Body Weight Gain %.

Biostatistics Studies

The data analysis was carried out with SPSS Inc. software (version 15.0). One-way ANOVA was used to study a significant difference between means of the groups with a significance level of P<0.05 when ANOVA analyses revealed differences among the rat groups, post-hoc analyses identified where the differences existed (Tukey HSD Test). All data are presented as ± Standard Deviation of Means (STDEV).

RESULTS

Body weight gain of rats

Table 3 Mean weight (g) changes in rats treated with normal fat level diets. Feed intake, body weight gain of rat groups were calculated and displayed in (Table 3). Feed mean intake (g) was significantly (P < 0.05) increased in all rat groups compared with NC. Body weight gain % has significantly (P < 0.05) increased in all rat groups compared to NC.

With a closer look at Table 3 rats fed on normal fat level (NC) had significantly (P < 0.05) lower mean of feed intake and body weight gain % compared with both HF and control groups (P < 0.05). PC shows most BWG% increase for rats (37.4). The combined group with Mg, Zn, and Cr at high level showing most decrease in BWG% (11.0).

Table 4: Effect of diets supplemented with Cr, Mg and Zn on total cholesterol and triacylglycerol in obese rats with diabetes.

Parameters Groups		mg/dl	
		Cholesterol	Triacylglycerol
Negative control group (NC)		80.6 ± 3.1^{g}	47.4 ± 1.9^{fgh}
Obese Diabetes group (PC)		165.3± 4.5^{a}	82.2± 5.0^{a}
Obese diabetes rat groups fed on normal diet and treated daily by daily oral doses of:	Distilled water group (DW)	137.2± 3.9^{b}	65.9± 4.5^{b}
	200-mg magnesium sulphate / (kg bw).	121.7± 3.3^{c}	56.0± 2.8^{ce}
	300 -mg magnesium sulphate / (kg bw).	109.2± 3.3^{e}	51.2 ± 2.8^{df}
	10 mg zinc chloride / (kg bw)	115.1 ± 2.6^{d}	53.2 ± 2.0^{de}
	20 mg zinc chloride / (kg bw)	105.4 ± 3.5^{e}	50.2 ± 3.1^{dh}
	100 µg chromium / (kg bw)	120.4 ± 2.3^{c}	54.1 ± 2.1^{cd}
	200 µg chromium / (kg bw)	108.1± 4.2^{e}	48.1± 3.7^{fgh}
	200-mg magnesium sulphate, 10 mg zinc chloride and 100 µg chromium / (kg bw)	106.4 ± 4.2^{e}	50.3 ± 4.0^{dg}
	300-mg magnesium sulphate, 20 mg zinc chloride and 200 µg chromium / (kg bw)	98.4 ± 6.1^{f}	48.8± 3.7^{fgh}

Significant differences at p < 0.05. Similar letters in the same column indicate non-significant differences.

Obese diabetes group (PC) showed elevated levels of cholesterol and triacylglycerol as found in Table 4. On the other hand, standard fat diets combined with trace minerals at low and high levels led to lowering cholesterol and triacylglycerol significantly at $P < 0.05$, similarly Cr administration at high level has a good effect on triacylglycerol. The rest of minerals had improved triacylglycerol and cholesterol levels at higher levels compared to low levels.

Lipoprotein estimation was improved in individual supplementation of diet with zinc (20mg) and at higher levels of combination of Mg, Zn and Cr in their diets. The only significant results lowered the VLDL-C to level approach from NC groups was chromium at higher level (200 µg) (Table 5).

Uric acid, urea nitrogen and creatinine were decreased significantly when diet supplemented with individual Zn at higher levels (20mg/ kg b.w). Continuing to look at the results in Table 6 indicate that combination at high levels showed reduction of uric acid urea nitrogen and creatinine than NC.

Combination of minerals at high levels gave good results for glucose and liver function enzymes. A significant reduction has been detected in rat groups supplemented with individual minerals at high levels (Table 7).

Results in Table 8 indicate that there are higher levels of thyroid hormones in obese rats specifically PC compared to NC. The combination of studied minerals at higher levels led to significant reduction of T_3 T_4 and TSH rat groups.

DISCUSSION

In the present study, we examined the association of dietary fat level supplemented with individual and combined minerals; Cr, Mg and Zn on liver and kidney functions, lipid profile and T3, T_4 and TSH levels in obese rats with experimental diabetes. Results indicate that there are good results among rat groups supplemented with minerals at individual and combined high levels.

It is known that there are several minerals that play an important role in modulating blood glucose and thyroid hormones. Experimental and clinical studies indicate that Zn and Cr deficiencies may predispose to glucose intolerance, diabetes mellitus, insulin resistance, and atherosclerosis (Roth and Kirchgessner 1991; Singh et al. 1995). However no large epidemiologic or animal studies have examined the combination of Zn, Cr and Mg on studied parameters.

We have noticed that supplementation with individual Zn at high level led to improvement of all parameters tested in this study specifically glucose levels. Studies indicate that Zn deficiency in conjunction with antioxidant deficiency causes insulin resistance. Zinc is a cofactor for superoxide dismutase which is an intracellular antioxidant enzyme. It has been suggested that as pancreatic beta cells have low antioxidative enzyme activities, they might be more sensitive to free radical damage which worsens if there is a coexisting zinc deficiency (Chen et al. 1998). There is some evidence that both zinc deficiency as well as excess can cause dyslipidemia and impair hepatic cholesterol synthesis and enhance oxidative stress (Subramanyam and Vijaya 1997). Zinc supplementation may inhibit platelet adhesiveness, increase fibrinolytic activity, and enhance healing processes which may be protective against coronary thrombosis and atherosclerosis. In this study zinc at high level (20 mg zinc chloride / kg b.w.) led to significant decrease of cholesterol. (Subramanyam and Vijaya 1997; Henning et al. 1996).

Table 5: Effect of supplemented diets with Cr, Mg and Zn on lipoprotein fractions in obese rats with diabetes.

Groups	Parameters	mg/dl		
		HDL-C	LDL-C	VLDL-C
Negative control group (NC)		47.8 ± 1.4^{a}	23.3 ± 2.4^{l}	9.4 ± 0.4^{fgh}
Obese diabetes group (PC)		30.4 ± 3.4^{j}	118.4± 1.4^{a}	16.4 ± 1.0^{a}
Obese diabetes rat groups fed on normal diet and treated daily by daily oral doses of:	Distilled water group (DW)	37.3±2.4^{efghi}	86.6 ± 1.0^{b}	13.1 ± 0.9^{b}
	200 -mg magnesium sulphate / (kg bw)	38.1 ± 2.3^{dh}	72.4 ± 1.0^{c}	11.2± 0.6^{ce}
	300 -mg magnesium sulphate / (kg bw)	40.7± 2.4^{cdf}	58.2 ± 1.2^{e}	10.2± 0.6^{df}
	10 mg zinc chloride / (kg bw)	39.1 ± 2.5^{cdg}	65.4 ± 1.2^{d}	10.6±0.4^{de}
	20 mg zinc chloride / (kg bw)	42.6 ± 4.2^{bc}	52.8± 1.3^{g}	10.0±0.6^{dh}
	100 µg chromium /(kg bw)	38.0± 2.0^{di}	71.9 ± 0.7^{c}	10.8± 0.4^{cd}
	200 µg chromium /(kg bw)	40.8 ± 4.5^{cde}	57.8± 1.8^{e}	9.6 ± 0.8^{fgh}
	200-mg magnesium sulphate, 10 mg zinc chloride and 100 µg chromium / (kg bw)	41.3± 2.8^{bd}	55.1± 0.8^{f}	10.1±1.0^{dg}
	300-mg magnesium sulphate, 20 mg zinc chloride and 200 µg chromium / (kg bw)	44.5 ± 4.3^{ab}	44.1 ± 2.2^{h}	9.7 ± 0.7^{fgh}

Significant differences at $P < 0.05$; Similar letters in the same column indicate non-significant differences; HDL-C = High density lipoprotein cholesterol. LDL = C : Low density lipoprotein cholesterol.

In our study combination of minerals gave good results related to biochemical parameters. On the other hand, the exact mechanism whereby zinc deficiency acts directly or by interaction with chromium, magnesium and antioxidants (The Indian Consensus Group for the Prevention of Diabetes 1997) in the pathogenesis of diabetes or CAD is not known.

There have been two randomized, placebo-controlled studies in Chinese patients with diabetes where chromium supplementation has had beneficial effects on glycaemia *(*Anderson et al. 1997; Cheng et al. 1999). However, the study populations may have had marginal baseline chromium status. In the first study (Anderson et al 1997), the chromium status was not evaluated either at baseline or after supplementation. Other smaller studies have also suggested a role for chromium supplementation in the management of diabetes (Ravina et al. 1995), glucose intolerance (Cefalu et al. 1999), gestational diabetes (Jovanovic et al. 1999), and corticosteroid-induced diabetes (Ravina et al. 1999). Results from these studies indicate that the dosage and formulation of chromium used significantly influences the outcome. In one study of patients with diabetes (Anderson et al. 1997), 1,000 µg/ day of chromium picolinate was

Table 6: Effect of supplemented diets with Cr, Mg and Zn on some kidney functions in obese rats with diabetes

Groups	Parameters	mg/dl		
		Uric Acid	Urea Nitrogen	Creatinine
Negative control group (NC)		1.6± 0.1^{h}	30.6 ± 2.3^{h}	0.6± 0.045^{g}
Obese diabetes group (PC)		2.8± 0.1^{a}	51.3 ± 4.1^{a}	1.2 ± 0.052^{a}
Obese diabetes rat groups fed on normal diet and treated daily by daily oral doses of:	Distilled water group (DW)	2.2± 0.2^{b}	43.1± 2.6^{b}	0.9± 0.086^{b}
	200 -mg magnesium sulphate / (kg bw)	2.1 ± 0.1^{c}	40.3± 2.4^{c}	0.8± 0.076^{c}
	300 -mg magnesium sulphate / (kg bw)	1.9 ± 0.1^{de}	38.5± 2.1^{cd}	0.8± 0.057^{cd}
	10 mg zinc chloride / (kg bw)	2.0 ± 0.1^{ce}	38.5± 1.3^{ce}	0.8±0.052^{ce}
	20 mg zinc chloride / (kg bw)	1.8± 0.1^{fg}	35.1 ± 2.7^{fg}	0.7 ± 0.056^{f}
	100 µg chromium /(kg bw)	2.1± 0.1^{cd}	40.6± 1.9^{bc}	0.9± 0.077^{c}
	200 µg chromium /(kg bw)	1.9 ± 0.1^{ef}	39.3 ± 2.4^{c}	0.8± 0.055^{c}
	200-mg magnesium sulphate, 10 mg zinc chloride and 100 µg chromium / (kg bw)	1.8 ± 0.1^{f}	36.0±2.1^{def}	0.7±0.056^{def}
	300-mg magnesium sulphate, 20 mg zinc chloride and 200 µg chromium / (kg bw)	1.7± 0.1^{gh}	32.7± 1.6^{gh}	0.7± 0.068^{f}

Significant differences at $P < 0.05$; Similar letters in the same column indicate non-significant differences.

Table 7: Effect of diets supplemented with Cr, Mg and Zn on serum glucose and liver enzymes of obese rats with diabetes

Groups	Parameters	Glucose mg/dl	U/l	
			AST	ALT
Negative control group (NC)		85.9 ± 3.1^{g}	68.3± 4.8^{e}	25.1± 2.7^{i}
Obese Diabetes group (PC)		198.8± 7.6^{a}	104.3± 6.8^{a}	53.2± 4.5^{a}
Obese diabetes rat groups fed on normal diet and treated daily by daily oral doses of:	Distilled water group (WG)	161.6± 6.6^{b}	85.3± 3.3^{b}	43.1± 4.0^{b}
	200 -mg magnesium sulphate / (kg bw)	147.2 ± 4.7^{c}	77.3± 3.1^{cd}	37.0± 2.4^{c}
	300 -mg magnesium sulphate / (kg bw)	136.4 ± 4.9^{d}	72.0± 4.2^{e}	31.1± 3.0^{efh}
	10 mg zinc chloride / (kg bw)	139.7± 3.1^{d}	79.1± 2.3^{c}	39.2 ± 3.0^{c}
	20 mg zinc chloride / (kg bw)	123.2± 4.3^{e}	71.9 ± 2.1^{e}	33.3± 2.3^{de}
	100 µg chromium /(kg bw)	141.9± 3.7^{cd}	73.1± 2.6^{de}	36.1± 2.8^{cd}
	200 µg chromium /(kg bw)	127.3± 5.4^{e}	70.0 ± 6.0^{e}	32.0± 2.8^{efg}
	200-mg magnesium sulphate, 10 mg zinc chloride and 100 µg chromium / (kg bw)	121.4± 7.3^{e}	71.6± 3.3^{e}	32.8± 3.2^{df}
	300-mg magnesium sulphate, 20 mg zinc chloride and 200 µg chromium / (kg bw)	109.1± 4.4^{f}	69.0± 4.0^{e}	28.8 ± 2.5^{gh}

Significant differences at $P < 0.05$; Similar letters in the same column indicate non-significant differences.

more effective than 200 µg/day. In the current study we used 200 µg/ Kg.b.w of chromium that reduced blood glucose levels more effectively compared to using 100 µg Kg.b.w. Similarly, in gestational diabetes 8 µg/day of chromium was more effective than 4 µg/day. In contrast, two well-designed studies in the U.S. (Sherman et al 1968; Abraham et al 1992) and two in Finland (Uusitupa 1984; Uusitupa et al 1992) failed to demonstrate any significant benefit of chromium supplementation in patients with diabetes. The latter studies used chromium chloride, which may not be as bioavailable as chromium picolinate. At the present time, benefit from chromium supplementation in rats with diabetes has been conclusively demonstrated.

The Institute of Medicine Food and Nutrition Board's DRIs found insufficient evidence to set an estimated average requirement for chromium. An adequate intake was determined based on estimated mean intakes. The adequate intake for adult men >51 years is 30 µg/day and for women >51 years is 20 µg/day. However, few serious adverse effects have been associated with excess intake of chromium from food supplements, and therefore a tolerable upper intake level has not been established.

A more recent placebo-controlled trial with a formulation of Zn and rabbit prostatic extracts found a significant reduction in HbA_{1c} in subjects randomized to the active treatment arm (Song et al. 1998). However, in that study, those randomized to the active treatment had higher baseline HbA_{1c} levels than those randomized to placebo.

From biochemical studies we understand that Zn is crucial for proper thyroid hormone metabolism. A moderate elevation of thyrotropin (TSH) concentrations, which is associated with triiodothyronine (T3) values in or slightly above the upper normal range, is frequently found in obese humans. These alterations seem rather a consequence than a cause of obesity since weight loss leads to a normalization of elevated thyroid hormone levels. In our study, thyroxin hormones were decreased significantly, specifically at higher level of individual supplementation of zinc (20 mg) that was in agreement with Kececi and Keskin (2002) whom supplemented diets of lambs and goats with zinc sulphate adjusted to 250 mg/kg for 12 weeks. They found that the levels of serum total thyroxin (T_4) and triiodothyronine (T_3) were lower in the lambs and goats of the Zn groups, except in the 4th week, as compared to those in the controls. Conversely, Maxwell and Volpe (2007) reported increase at 4 months, total T_3 concentrations in two zinc-deficient female college students (ZD1 and ZD2) that were supplemented with 26.4 mg/day of zinc (as zinc gluconate), while all thyroid hormone concentrations increased in ZD2. The same trend has been obviously reported when minerals are combined at higher levels in rats' diets.

Table 8: Effect of diets supplemented with Cr, Mg and Zn on T_3, T_4 and TSH of obese rats with diabetes

Groups	Parameters	T_3 Ng/dl	T_4 µg/dl	TSH µlu/ml
Negative control group (NC)		125.2 ± 3.1^{g}	6.9 ± 0.2^{h}	0.102±0.013^{i}
Obese Diabetes Group (PC)		147.8 ± 5.3^{a}	8.9 ± 0.2^{a}	0.169±0.010^{a}
Obese diabetes rat groups fed on normal diet and treated daily by daily oral doses of:	Distilled water group (WG)	139.4 ± 1.6^{b}	8.7 ± 0.2^{ab}	0.153±0.007^{b}
	200 -mg magnesium sulphate / (kg bw)	134.9 ± 1.6^{cd}	8.4 ± 0.3^{bc}	0.145±0.010^{bc}
	300 -mg magnesium sulphate / (kg bw)	133.5± 2.6^{cde}	8.3± 0.1^{cd}	0.140±0.006^{cd}
	10 mg zinc chloride / (kg bw)	135.1 ± 2.8^{cd}	8.1 ± 0.2^{de}	0.127 ± 0.004^{ef}
	20 mg zinc chloride / (kg bw)	130.4± 2.8 ^{ef}	7.9 ± 0.2^{ef}	0.123± 0.003^{fg}
	100 µg chromium /(kg bw)	137.1 ± 2.5^{bc}	8.3± 0.2^{cd}	0.138± 0.1^{cd}
	200 µg chromium /(kg bw)	131.9 ±3.1^{def}	7.9 ± 0.2^{ef}	0.132 ± 0.1^{de}
	200-mg magnesium sulphate, 10 mg zinc chloride and 100 µg chromium / (kg bw)	132.5 ± 2.9^{de}	7.7± 0.3^{f}	0.115± 0.1^{gh}
	300-mg magnesium sulphate, 20 mg zinc chloride and 200 µg chromium / (kg bw)	128.7±1.2^{f}	7.4 ±0.164^{g}	0.111 ±0.005^{h}

Significant differences at $P < 0.05$; Similar letters in the same column indicate non-significant difference; T_3 = Triiodothytronine; T_4 = Thyroxine; TSH = thyroid-stimulating hormones

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