Pakistan J. Zool., vol. 43(2), pp. 379-386, 2011.

Relationship of Thyroid Hormones with Serum Fasting Insulin and Insulin Resistance in Euthyroid Glycemic Anomalies

Tasnim Farasat,*[1] Abdul Majeed Cheema[2] and Muhammad Naeem Khan[2]
[1]*Department of Zoology, Lahore College for Women University, Lahore*
[2]*Department of Zoology, University of the Punjab, Quaid-i-Azam Campus, Lahore.*

Abstract.- A total of 277 euthyroid subjects, diagnosed as normal glucose tolerant, impaired glucose tolerant, and type 2 diabetic Pakistani subjects were sampled and analyzed for metabolites and data was worked out to investigate the relationship of thyroid hormones and insulin resistance in the glycemic anomalies. Oral glucose tolerant test (OGTT) was employed to confirm the glycemic status. The subjects were categorized using Diabetes Expert Committee criteria (2003). Thyrotropin (TSH), total triiodothyronine (TT_3), total thyroxin (TT_4) and insulin were assessed by enzyme linked immunoassays (ELISA). Fasting plasma glucose and HbA1c were measured by glucose oxidase and low pressure cat ion exchange chromatography. Homeostasis model of assessment (HOMA-IR) was employed to assess the level of insulin resistance. Anthropometric measurement and habits were recorded. Younger glucose intolerant subjects were highly hyperinsulinemic and insulin resistant as compared to the diabetic group ($p<0.05$).Serum concentration of TT_3 was significantly low in IGT and diabetic group as compared to NGT ($p<0.05$). Serum concentration of TSH was significantly higher in IGT and diabetic group. A significant inverse correlation of fasting serum insulin and insulin resistance with T_3 prevailed in the nomal glucose tolerant subjects ($r= -0.45$., $r=-.345$ $p<0.05$). In multiple regression analysis when insulin was a dependent variable TT_4 and TSH were significant predictors of insulin secretions in IGT and diabetic groups respectively $p<0.05$. The homeostatitic relationship of insulin and T_3 has been lost with the parallel development of insulin resistance and hyperglycemia in glycemic anomalies. The ratio of insulin and T_3 is important in glucose homeostasis.

Key words: Impaired glucose tolerance, diabetes type 2, T3, insulin, TSH, thyroid hormones.

INTRODUCTION

Diabetes and thyroid disease are common in our population. There is a continuous association of thyroid hormones and insulin in glucose metabolism (Dimitriadis and Raptis, 2001; Lacobellis *et al.*, 2005; Chakarabarti, 2007). Glucose homeostasis is achieved by an extremely complex mechanism involving, along with food intake, the regulation of insulin secretion and its action at a target tissue level. Thyroid hormone (TH) action has long been recognized as an important determinant of glucose homeostasis (Dimitriadis *et al*., 1985; Weinstein *et al*., 1991, 1994; Torrance *et al.*, 1997). IR is defined as an inability of insulin to produce its biological effects at physiological concentrations and is a cardinal feature of T2DM (Harris *et al*., 1998; Ferrannini, 2004). It is characterized by the impaired ability of insulin to inhibit hepatic glucose output and to stimulate glucose uptake into skeletal muscle. Most subjects at risk of cardiovascular disease (CVD) are euthyroid (Roos *et al*., 2007). Many studies revealed that T3 and insulin both stimulate the expression of hexokinases and glycogen synthase which are respectively responsible for uptake and disposal of glucose via formation of glucose-6 phosphate and glucose-1 phosphate (Hoppner and Seitz, 1989; Granner and Pikis, 1990; Kim *et al.*, 2002; Chidakel *et al.*, 2005). In some study population it was found that IR modifies the relationship between thyroid function and insulin sensitivity (Kim *et al*., 2002; Chubb *et al.*, 2005). At a molecular level, microarray studies performed on mice liver have demonstrated that most of the enzymes involved in gluconeogenesis are positively regulated by thyroid hormone (Feng *et al.*, 2000). It is known that TH can stimulate the expression and activate a number of proteins that are candidates for regulating insulin sensitivity (Klieverik *et al.*, 2009). It was reported by Ledda *et al. (*2005) that T3 is a powerful inducer of pancreatic acinal cell proliferation in rodents. Ortega *et al.* (2008) demonstrated that T3 concentrations were positively associated with

* Corresponding author: tasnimfarasat@hotmail.com
0030-9923/2011/0002-0379 $ 8.00/0

insulin secretions and T3 may play a role in the insulin secretions . Glucose intolerance (IGT) is an intermediate category between normal glucose tolerance (NGT) and diabetes. Despite the mild glucose homeostasis it is well documented that IGT subjects are at increased risk of developing macrovascular complications as compared to the diabetics (Oberlinner *et al.,* 2008). This study was focused on euthyroid impaired glucose tolerant and newly diagnosed type 2 diabetic subjects to investigate the relationship of thyroid hormones and insulin secretions in glucose homeostasis.

MATERIALS AND METHODS

Clinical facility

A total of 277 Euthyroid and Hyperglycemic Subjects were screened during the year 2005-2006 at Amin Hyat Memorial Trust for Diabetes and Hypertension. This is an outpatient charity hospital located in a low income area near Multan Road, Lahore. The hospital is managed by highly qualified team of physicians and it is sponsored by Saith Abid.

This unit supports the primary care of Diabetic patients by providing clinical consultation and basic laboratory assessment besides free medication. The hospital management has complete computerized record of all patients with different categories of type 1 and type-2 diabetes mellitus and impaired glucose tolerance. For every visiting patient a detailed questionnaire including medical history and habits has been designed according to WHO criteria.

Experimental planning

In this study the populations of clinically normal, euthyroid, diagnosed as glycemically intolerant and diabetics were sampled and analyzed for metabolites and data was worked out to investigate the relationship of thyroid activity in glucose homeostasis. To assess the thyroid status in the glycemic anomalies serum levels of insulin, thyrotropin (TSH), Triiodothyronine (TT3), Thyroxine (TT4), were measured by sensitive enzymes linked Immunoassay (ELISA) on fully automated device (CODA). A total number of 277 selected subjects of either sex with age 35-68 were included in the study. The subjects were divided into three groups according to their glucose levels as control group (n = 90, with fasting plasma glucose 74-100 mg/dl); IGT group (n=92) (with fasting plasma glucose 100-125 mg/dl) and newly diagnosed Diabetic group (n=95) fasting plasma glucose 126-250 mg/dL.

On the study day, the subjects attended the clinic in a fasting state. Screening questionnaire revealed information on the condition. In every single patient the specific clinical reason for any pharmacological treatment was recorded. Clinical documentation represented by the hospital records was found in already diagnosed diabetic patients. Our screening approach was specifically aimed at identifying the impaired glucose tolerants and diabetic subjects with recent onset of the disease (duration of disease since its diagnosis less than two years). Oral glucose tolerant test (OGTT) was employed to confirm the glycemic status among the subjects under the expert supervision of hospital management. Those subjects with thyroid dysfunction, hepatic and renal disorder or cardiac impairment were excluded. Among the diabetic subjects, 50% were undiagnosed previously, while IGT subjects were the first or 2nd degree relatives of the diabetic subjects, who most of the time accompanied them; previously diagnosed diabetic subjects included 60% female, 40% male, all in the age group between 40-50 years.

Biochemical assessment

Glucose was measured by the glucose oxidase method on venous whole blood immediately deproteinized with perchloric acid on chemistry Analyzer (Hitachi, 902). The obtained values were transformed into plasma glucose values in order to calculate the insulin resistance (Mathew *et al*., 1985). HOMA-IR was derived from simultaneous FPG (mmol/l) and fasting plasma insulin (µIU/l) measurements, while HbA1c was assessed on whole blood (Diastat Analyzer, Bio Rad USA).

Inclusion criteria were strictly followed. They were all euthyroid with normal thyroid function. Euthyroidism was defined by thyroid hormones concentrations in normal reference range. It was 0.8-5.0 µIU/ml for TSH, for TT3 range was 0.52-1.85 ng/ml and for TT4 reference range 4.4-10.8

µg/dl for males and 4.8-11.6 for female. To avoid the influence of confounding factors, the following subjects were excluded from the study: smokers, subjects taking any kind of medication or drug, patients with stroke or heart diseases. Respecting the above exclusion criteria we selected 187 subjects (both genders) aged 35-68 years and in these subjects no associated illness had been reported. Both IGT and diabetics had positive family history of diabetes. All were non-smokers, 40% were sedentary and 60% were moderate. They were not taking any kind of medicine.

Insulin resistance

Homeostasis model assessment was employed to estimate insulin resistance from simultaneous FPG and fasting plasma insulin levels

$$\text{HOMA} - \text{IR} = \frac{\text{Fasting Insulin (µIU/ml) x Fasting Glucose (µIU/ml)}}{22.5}$$

Insulin resistance was defined as the level of homeostasis model assessment for insulin resistance (HOMA-IR) greater than 3.1 (Matthews *et al.*, 1985).

Immunoassays

Hormones were assessed by ELISA technique with the fully automated compact open device automation (CODA) in ELISA Laboratory of Zoology Department at Lahore College for women University, Lahore. Commercially available Monobind Inc. ELISA Kits (CA 92630 USA) were used for reliability of assay. Total circulating T3 and T4 were estimated by enzyme linked immunoassay following the protocol provided in the commercially available ELISA Kits, supplied by Monobind CA 92630, USA. A set of quality control sera was also used with each assay to monitor the procedure. Insulin and TSH were assessed by Immunoenzymometric Assay (Type 3), which included high affinity and specificity of antibodies (conjugated and immobilized) and native antigen. A sandwich complex is formed, while TT3 and TT4 were assessed by competitive enzyme immunoassay (type 5), which included immobilized antibody, enzyme antigen conjugate and native antigen. A competition reaction results between the enzyme conjugate and the native antigen for a limited number of antibody combining sites immobilized on the well. Horse Radish Peroxidase Enzyme was used. The apparatus was fully automated. Protocol was entered in the software with all the steps according to instructions of manufacturers on the literature that was available with the Kit. All assays were performed on the same serum samples and on the same apparatus to avoid any discrepancies. Standards and quality control were run within each assay for monitoring assay performance.

Statistical analysis

Statistical analysis was performed using the SPSS (version 13.0) software. All Results are expressed as means ± SEM. Pearson correlation was used to find relationship among the variables and ANOVA with post hoc was applied to find significance among the groups. Stepwise multiple Regression analysis was employed to study the joint effect of variables on the fasting plasma insulin level.

RESULTS

Anthropometric and biochemical characteristics of the studied population are presented in Table I .Marked hyperinsulinemia has been shown in the glucose intolerant and T2DM subjects as compared to the controls ($P<0.05$).The mean value of serum TT3 in the control IGT and diabetic group was 0.95±0.06 ng/ml, 0.70±0.02 ng/ml and 0.61±0.01 ng/ml, respectively. The levels of triiodiothyronine (TT3) were significantly different among control IGT and diabetic ($P<0.05$). The means values of serum TT4 concentration in the control group was 4.85±0.35 µg/dl in IGT group 8.52±0.24 µg/dl and in diabetic group 7.98±0.12 µg/dl. The levels of TT4 were significantly different among the three groups ($P<0.05$).

The serum TSH concentration in the control, IGT and diabetic groups were 1.82±0.19 µIU/ml, 4.32±0.20 µIU/ml and4.89±0.70 µIU/ml, respectively. TSH difference between the three groups was significant ($P<0.05$) (Table I).

The descriptive statistics showed that the mean value of insulin resistance in control group

Table I.- Anthropometric and biochemical characteristics of the studied population.

Parameters	Control Mean ± SE (n=90)	IGT (Mean ± SE) (n=92)	Diabetes Mean ± SE (n=95)	P-value
Age (year)	50.73±0.94	49.93±1.06	53.21±0.81	0.026*
Body mass index (BMI) (Kg/m^2)	24.00±0.40	31.18±0.49	30.77±0.53	0.001**
Fasting plasma glucose (FPG) (mg/dl)	87.42±1.38	116.04±0.89	162.36±4.52	0.001**
HbAIc (%)	5.76±0.05	6.54±0.07	8.66±0.15	0.001**
Insulin (μIU/ml)	9.44±0.68	51.73±1.53	23.20±0.71	0.001**
TT_3 (ng/ml)	0.95±0.06	0.69±0.02	0.68±0.01	0.024*
TT_4 (μg/dl)	4.85±0.35	8.52±0.24	7.98±0.12	0.026*
TSH (μIU/ml)	1.87±0.18	4.32±0.20	4.39±0.70	0. 03*
Insulin resistance (HOMA IR)	2.02±0.14	$14.86±0.48^{a,c}$	9.41±0.32	0.001**

Data expressed: mean ± SEM * = P<0.05: ** = P<0.01

Table II.- Correlation analysis among different parameters of control, IGT and diabetics in the studied population.

Parameters	Groups	BMI		FPG		HbA1c		Insulin		IR	
		r	p	r	p	r	p	r	p	r	p
TSH	Control	0.36	0.04*	-0.11	0.55	-0.18	0.33	0.25	0.16	0.23	0.20
	IGT	-0.39	0.03*	-0.12	0.39	0.10	0.47	-0.33	0.03*	-0.37*	0.02*
	Diabetics	-0.05	0.62	0.09	0.41	0.03	0.76	-0.13	0.23	-0.05	0.67
TT_3	Control	0.08	0.65	0.26	0.14	0.12	0.52	-0.50	0.03**	-0.37	0.01**
	IGT	-0.10	0.48	-0.14	0.31	-0.02	0.88	-0.23	0.04*	-0.26	0.045*
	Diabetics	0.01	0.92	-0.14	0.18	-0.11	0.30	0.06	0.56	-0.05	0.67
TT_4	Control	-0.00	0.99	0.33	0.06	0.15	0.39	-0.44*	0.01*	-0.37	0.03
	IGT	0.11	0.41	-0.13	0.36	-0.28	0.04*	0.24	0.08	0.18	0.18
	Diabetics	0.05	0.66	-0.07	0.48	-0.02	0.85	0.06	0.55	-0.38*	0.024*

r = Correlation coefficient Significance: * = P<0.05 ** = P<0.01

was 1.89±0.14, in IGT 14.86±0.48 and in the diabetic group 9.41±0.32. HOMA-IR among the three groups *i.e.*, control group, IGT group and diabetic group was significantly different (P<0.01) (Table I). Insulin resistance was higher in IGT group as compared to diabetics. Gender differences were not significant (data not shown).

Correlation analysis

In this study, we have analyzed the correlation between thyroid hormones, fasting serum insulin levels and insulin resistance among euthyroid glycemic anomalies and NGT subjects.TT3 had significant and positive correlation with TT4 (r=0.700, r=0.577) in control and diabetic subjects respectively (P<0.01), while relationship was non-significant in IGT subjects. Correlation of insulin with TSH was significant in IGT and T2DM subjects (P<0.05).A significant negative correlation of serum insulin and IR with TT3 in NGT subjects was observed. While this relationship was positive in the IGT and type 2 DM subjects. Thyrotropin levels correlated positively with BMI in all the studied groups (P<0.05).

In multiple regression analysis TSH, TT4 contributed significantly to the variance of fasting insulin in IGT and diabetic subjects (Table III a,b,c).

DISCUSSION

The present study was undertaken to find the functional status of the thyroid and its regulatory hormones on homeostasis of glucose.Demographic data of the study population revealed that in the diabetic and IGT groups, females were younger as compared to males. The main findings of the hormonal analysis in the selected population revealed that insulin concentration was significantly

Table III.- Stepwise linear model regression coefficient for factors associated with serum insulin concentration in the control group. Adjusted R^2 = 0.141

Model	Control Group	Unstandardized Coefficients		Standardized Coefficients	t	Significant
		B	Std. Error	Beta		
1	(Constant)	15.171	2.574		5.894	0.001**
	TT_3	-8.474	2.983	-0.401	-2.841	0.007*

a. Dependent variable: Insulin; Determinants of insulin concentration: TT_3
Stepwise criteria: Probability of F to enter $\leq$ 0.05 and probability of F to remove was $\geq$ 0.10
Significance: * = P<0.05; ** = P<0.01

Table IIIb.- Stepwise linear model regression coefficients for factors associated with serum insulin concentration in the IGT group. Adjusted R^2 = 0.81

Model	IGT Group	Unstandardized Coefficients		Standardized Coefficients	t	Significant
		B	Std. Error	Beta		
1	(Constant)	32.578	7.687		4.238	0.001**
	TT_4	2.112	0.882	0.313	2.395	0.020*
2	(Constant)	-28.728	24.042		-1.195	0.238*
	TT_4	2.293	0.837	0.339	2.739	0.008*
	Fasting PG	0.515	0.192	0.332	2.675	0.010*

a. Dependent Variable: Insulin Beta estimates partial relation coefficient
b. Independent variables: FPG, HbA1c, TT_3, TT_4, TSH.(age, BMI and IR not included).
Determinants of Insulin concentration: TT_4, FPG.
Stepwise criteria: Probability of F to enter was $\leq$ 0.05 and probability of F to remove was $\geq$ 0.10.
Significance: * = P<0.05; ** = P<0.01

Table IIIc.- Stepwise linear model regression coefficient for factors associated with serum insulin concentration in the diabetic group. Adjusted R^2 = 0.961

Model	Diabetic Group	Unstandardized Coefficients		Standardized Coefficients	t	Significant
		B	Std. Error	Beta		
1	(Constant)	26.821	1.523		17.612	0.001*
	TSH	-1.761	-.732	-0.246	-2.406	0.018
2	(Constant)	24.500	1.721		14.239	0.001*
	TSH	-1.978	0.714	-0.276	-2.770	0.007*
	FPG	0.041	0.016	0.261	2.621	0.010
3	(Constant)	32.024	3.988		8.030	0.001*
	TSH	-1.833	0.705	-0.256	-2.602	0.011*
	FPG	0.040	0.015	0.257	2.626	0.010*
	HbA1c	-0.895	0.430	-0.203	-2.083	0.040*

a. Dependent variable: Insulin; Constant: TSH
Predictors of serum insulin concentration: TSH, FPG, HbA1c
Independent variables: FPG, HbA1c, TT_3, TT_4, TSH.
Stepwise criteria: Probability of F to enter was $\leq$ 0.05 and probability of F to remove was $\geq$ 0.10.
Significance: * = P<0.05; ** = P<0.01

higher in the IGT and diabetic groups as compared to the controls (P<0.05). A significantly lower serum TT_3 levels and higher levels of TSH and TT4 in the IGT and diabetic subjects as compared to

control were observed (<0.05). Serum T_3 concentration towards lower limit of normal and serum T_4 concentration towards higher limit of normal results in progressive increase in T_3 fraction and may depend on it to keep euthyroidism. In the current study there was a significant inverse association of fasting serum insulin with serum TT_3 concentration in the normal subjects.

It is well documented that serum T_3 and insulin levels both reciprocally regulate the rate of glycolysis and storage of glucose both at the molecular as well as at the physiological levels (Kim *et al*., 2002). T_3 may directly stimulate the uptake of glucose. Inhibition of entry of insulin and glucose due to the defective network of membrane receptors for hormonal signal transduction within the membrane via abnormalities of G proteins, cAMP, GLUT-2 and GLUT-4 of the membranes (Meyers *et al*., 1997; Pan *et al*., 1997) results in hyperglycemia. T_3 is responsible not only for stimulation of cAMP, which is in turn responsible for gluconeogenesis and reciprocal action against insulin activity, but also for the physiological uptake of glucose and insulin as well as the expression of glycolytic enzymes (Gilman, 1984; Granner and Pikis, 1990). Insulin opposes the effects of several catabolic hormones, such as T_3, that increase cAMP and regulate lipid and carbohydrate metabolism in adipose tissue and liver (Kim *et al*., 2002), therefore to achieve normoglycemia the optimal levels of the rate-limiting enzymes such as HK, glycogen synthase, PFK and pyruvate dehydrogenase, respectively, require the successful collaboration between T_3 and insulin (Weinstein *et al*., 1991; Granner and Pikis, 1990).

In the IGT and T2DM subjects lower levels of T_3 and higher levels of insulin failed to maintain normoglycemic condition and this further enhanced the hyperinsulinemia. Consistent hyperglycemia in the hyperinsulinemic and IR subjects might be attributable to this disturbed balance between insulin and T_3, which fail to maintain normal glycemia. The results of the present study show that the homeostasis relationship of insulin and T_3 is gradually lost with the parallel development of IR and insufficiency of insulin to the cells. These results are in line with the two previous studies when it was observed that insulin resistance with the hyperglycemia, hyperinsulinemia, and the altered natures of skeletal muscle fibers with lower ratio of oxidative to glycolytic enzymes, reduced the glucose disposal via reduced oxidation of glucose (Shmiokawa *et al*., 1997; Crunkhorn and Petti, 2008). In this study IGT subjects are hyperinsulinimic and insulin resistant. Chubb *et al*. (2005) demonstrated that at low insulin sensitivity relatively minor differences in TSH are associated with marked changes in lipid levels that is a risk factor for cardio-vascular disease. The correlation was stronger with T3 and TSH as compared to T4. Total T3 is the biologically active hormone in the tissues and in our study it has been associated with insulin resistance in NGT subjects.

In skeletal muscle, IR may be caused by defects in glucose transport, which result from impairments in the translocation, fusion, or exposure and activation of GLUT-4 glucose transporters (Cline *et al*., 1999). These abnormalities in GLUT-4 translocation in muscle appear to result from defects in intracellular signaling which leads to hyperglycemia and insulin resistance. Although serum concentrations of thyroid hormones lies in normal range minor differences seem to be important as already reported in previous studies (Davis *et al*., 2001; Andersen *et al*., 2007). In summary we conclude that thyroid hormones are associated with insulin resistance in euthyroid range. Our data showed that thyroid hormones are related with indices of glucose metabolism.

This highlights the changes in TSH levels in normal range are significantly associated with insulin resistance. Our results indicate that elevated T4 and TSH are related to abnormal glycemic levels and insulin resistance based on our study we postulate that elevation of TSH and T4 indicate involvement of thyroid gland in insulin resistance.

REFERENCES

ANDERSEN, S., PEDERSEN, K.M., BRUUN, N.H. AND LAURBERG, P., 2007. Narrow individual variations in serum T4 and T3 in normal subjects: A clue to the understanding of subclinical thyroid disease. *J. clin. Endocrinol. Metabol.*, **87**: 1068-1072.

CHAKRABARTI, S., GURIA, S., SAMANTA, I. AND DAS, M., 2007. Thyroid dysfunction modulates glucoregulatory mechanism in rat. *Indian J. exp. Biol*., **45**: 549-553.

CHIDAKEL, A., MENTUCCIA, D. AND CELI, F.S., 2005. Peripheral metabolism of thyroid hormone and glucose homeostatis. *Thyroid*, **15**: 899-904.

CHUBB, S.A.P., DAVIS, W.A. AND DAVIS, T.M.E., 2005. Interactions among thyroid function, insulin sensitivity and serum lipid concentrations: the Fremantle diabetes study. *J. clin. Endocrinol. Metab.*, **90**: 5317-5320.

CLINE, G.W., PETERSEN, K.F., KRSSAK, M., SHEN, J., HUNDAL, R.S., TRAJANOSKI, Z., INZUCCHI, S., DRESNER, A., ROTHMAN, D.L. AND SHULMAN, G.I., 1999. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. *N. Engl. J. Med*., **34**: 240-246.

CRUNKHORN, S. AND PATTI, M.E., 2008. Links between thyroid hormone action, oxidative metabolism and diabetes risk? *Thyroid*, **18**: 227.

DAVIS, F.B., THAYER, K. AND COLBORN, T., 2001. significant effects of mild endogenous Hormonal changes in humans: considerations for low dose testing. *Environ. Hlth. Pers.*, **109**: 21-25.

DIMITRIADIS, G. AND RAPTIS, S.A., 2001. Thyroid hormone excess and glucose intolerance. *Exp. Clin. Endocrinol. Diabetes*, **109**: S225-S239.

DIMITRIADIS, G., BAKER, B., MARSH, H., MANDARINO, L., RIZZA, R., BERGMAN, R., HAYMOND, M. AND GERICH, J., 1985. Effect of thyroid hormone excess on action, secretion and metabolism of insulin in humans. *Am. J. Physiol*., **248**: E593-601.

FENG, X., JIANG, Y., MELTZER, P. AND YEN, P.M., 2000. Thyroid hormone regulation of hepatic genes *in vivo* detected by complementary DNA microarray. *Mol. Endocrinol*., **14**: 947-955.

FERRANNINI, E., 2004. Hyperinsulinemia and insulin resistance. In: *Diabetes: A fundamental and clinical text*, 3rd edn. (eds. D. Le Roith, S.I. Taylor and J.M. Olefsky).

GILMAN, A.G., 1984. G-proteins and dual control of adenylate cyclase. *Cell*, **36**: 577-579.

GRANNER, D.K. AND PIKIS, S., 1990. The genes of hepatic glucose metabolism. *J. biol. Chem*., **265**: 10173-10182.

HARRIS, M.I., FLEGAL, K.M., COWIE, C.C., EBERHARD, M.S., GOLDSTEM, D.E., LITTLE, R.R., WIEDMEYER, H.M. AND BYRD-HOLT, D.D., 1998. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The 3rd National Health and Nutrition Examination Survey, 1988-1994. *Diabetes Care*, **4**: 518-524.

HOPPNER, W. AND SEITZ, H.J., 1989. Effect of thyroid hormones on glucokinase gene transcription in rat liver. *J. biol. Chem*., **264**: 20647

KLIEVERIK, L.P., JANSSEN, S.F., VAN RIEL, A., FOPPEN, E., BISSCHOP, P.H., SERLIE, M.J., BOELEN, A., ACKERMANS, M.T., SAUERWEIN, H.P., FLIERS, E. AND KALSBEEK, A., 2009. Thyroid hormone modulates glucose production via a sympathetic pathway from the hypothalamic paraventricular nucleus to the liver. *Proc. natl. Acad. Sci*., **106**: 5966-5971.

LACOBELLIS, G., RIBAUDO, M.C., ZAPPATERRENO, A., LANNUCCI, C.V. AND LEONETTI, F., 2005. Relationship of thyroid function with body mass index, leptin, insulin sensitivity and adiponectin in euthyroid obese women. *Clin. Endocrinol*., **62**: 487-491.

LEDDA-COLUMBANO, G.M., PERRA, A., PBIRI, M., MOLOTZU, F. AND COLUMBANO, A., 2005. Induction of pancreatic acinar cell proliferation by thyroid hormone. *J. Endocrinol*., **185**: 393-399.

MATTHEWS, D.R., HOSKER, J.P., RUDENSKI, A.S., NAYLOR, B.A., TREACHER, D.F. AND TURNER, R., 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, **28:** 412-419.

MEYERS, D.S., SKWISH, S., KENNETH, E.J., KIENZLE, D.B. AND ARBEENY, C., 1997. 3-adrenergic receptor-mediated lipolysis and oxygen consumption in brown adipocytes from cynomologus monkeys. *J. clin. Endocrinol. Metab*., **82**: 395-401.

OBERLINNER, C., NEUMANN, S.M., OTT, M.G. AND ZOBER, A., 2008. Screening for pre-diabetes and diabetes in the workplace. *Occup. Med. (Lond.)*, **58:** 41-45.

ORTEGA, E., KOSKA, J., PANNACCIULLI, N., BUNT, J.C. AND KRAKOFF, J., 2008. Free triiodothyronine plasma concentrations are positively associated with insulin secretion in euthyroid individuals. *Eur. J. Endocrinol*., **158**: 217-221.

PAN, D.A., LILLIOLJA, S., KRIKRTOS, A.D., MILLER, M.R., BAUER, L.A. AND BORGADUS, C., 1997. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes*, **46**: 983-988.

ROOS, A., BAKKER, S.J., LINKS, T.P., GANS, R.O. AND WOLFFENBUTTEL, B.H., 2007. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. *J. clin. Endocrinol. Metab*., **92**: 491-496.

SHIMIZU, Y. AND SHIMAZU, T., 2002. Thyroid hormone augments $GLUT_4$ expression and insulin-sensitive glucose transport system in differentiating rat brown adipocytes in culture. *J. Vet. Med. Sci*., **64**: 677-681.

SHIMOKAWA, T., KATO, M., SHIODUKA, K., IRIE, J. AND EZAKI, O., 1997. Effect of triiodothyronine on muscle cell differentiation and blood glucose level in hyperglycemic KK mice. *Biochem. biosphys. Res. Commun.*, **235**: 790-793.

THE EXPERT COMMITTEE ON THE DIAGNOSIS AND CLASSIFICATION OF DIABETES MELLITUS, 2003. Follow up report on the diagnosis of diabetes mellitus. *Diabetes Care*, **26**: 3160-3167.

TORRANCE, C.J., DEVENTE, J.E., JONES, J.P. AND DOHM, G.L., 1997. Effects of thyroid hormone on

GLUT4 glucose transporter gene expression and NIDDM in rats. *Endocrinology*, **138**: 1204-1214.

WEINSTEIN, S.P., WATTS, J. AND HABER, R., 1991. Thyroid hormone increases muscles/fat glucose transporter gene expression in rat skeletal muscle. *Endocrinology*, **129**: 455-464.

WEINSTEIN, S.P., O'BOYLE, E. AND HABER, R.S., 1994. Thyroid hormone increases basal and insulin-stimulated glucose transport in skeletal muscle. The role of GLUT4 glucose transporter expression. *Diabetes*, **43**: 1185-1189.

(Received 22 June 2010, revised 20 September 2010)