Association of serum Leptin and Adiponectin with Atherosclerosis in obese and non-obese Type 2 Diabetes Mellitus patients

Mohga S Abdalla[1], Hayat M Sharada[1], Ashraf I Amin[2], Nervana Samy[3], Magda Sayed[3],Esmat Ashour[3] and Elham M Youssef-Elabd[3.]
[1]Biochemistry Dept, Faculty of Science, Helwan University, Egypt;
[2]Clinical Pathology Dept, National Institute of Diabetes & Endocrinology, Cairo, Egypt.
[3]Biochemistry Dept, National Research Centre, Dokki, Giza, Egypt
Nervana91@hotmail.com

Abstract: Obesity is a major risk factor for insulin resistance, type 2 diabetes, heart disease, and many other chronic diseases The current study was designed to investigate the endogenous mechanism by which obesity may increase the risk of CVD by examining whether serum adiponectin, Leptin or insulin mediate the association of obesity and type2 diabetes and cardiovascular risk factors in Egyptian adult patients. **Patients and Methods:** This study included 82 subjects, 30 patients suffering from type 2 diabetes and 52 patients suffering from type 2 diabetes together with coronary artery disease (CAD) together with another group having CAD without diabetes. They were classified according to their body mass index (BMI) into obese and non-obese groups, also 25 healthy volunteers were considered as controls. All patients were subjected to anthropometric assessment and laboratory determination of serum Adiponectin, Leptin, insulin and glucose. Insulin resistance was established by homeostasis model assessment (HOMA-IR) Differences in clinical or laboratory parameters among groups were compared by using one-way ANOVA test. **Results** revealed highly significant decrease in Adiponectin levels and highly significant increase in serum Leptin in non obese groups (G1 (T2D), G2 (CAD) and G3 (T2D+ CAD) as compared to controls. However, there were no statistical variations between non obese groups when compared to each others. HOMA-IR showed highly significant increase in non obese groups as compared to both controls and each other. Also, the results showed high significant decrease in Adiponectin and highly significant increase in Leptin in obese groups (G4 (T2D), G5 (CAD) and G6 (T2D+CAD) when compared to controls. However, there were no statistical variations between obese groups when compared to each others as regard Adiponectin, while Leptin showed statistical increase between (G4) and (G5) groups when compared to each others, HOMA-IR showed highly significant increase in the two obese groups only (G4 and G6) as compared to controls, while there was no significant variation in (G5) when compared to controls. Moreover, there was a significant increase in all obese groups when compared to each other. Also, there was significant correlation between serum Adiponectin and Leptin in obese DM patients. **Conclusion:** The coexistence of correlation between serum leptin and Adiponectin levels in addition to increase of serum leptin and decrease serum Adiponectin levels in obese DM patients in the current study; support the hypothesis of their susceptibility to atherosclerosis. [Journal of American Science 2010;6(5):153-164]. (ISSN: 1545-1003).

Keywords: Type2 Diabetes, Cardiovascular disease, Adiponectin, Leptin, HOMA-IR

1. Introduction

Obesity is a major risk factor for insulin resistance (IR), type 2 diabetes, heart disease and many other chronic diseases .These associations are influenced by adipose tissue (AT) distribution (Marcus et al., 1999). IR is characteristically more severe in T2DM than in similarly obese non-diabetics, but whether this difference is related to differences in body composition is unclear (Azuma et al., 2007; Marinou et al., 2009).

Type 2 diabetes mellitus (T2D) is considered one of the major metabolic diseases of 21st century (Kowalska,2007, Thévenod 2008). The excessive intake of food, sedentary life style and lack of physical activity are responsible for the growing epidemic of obesity, together with the increasing rate of T2D in many parts of the world (Zimmet, et al., 2001) The main burden of T2D is connected with development of vascular complications, which are a consequence of accelerated atherogenesis (Cooper et al., 2001; Salas-Salvadó et al., 2007).

Atherosclerotic cardiovascular complications are the major causes of morbidity and mortality in type 2 diabetic patients. Cardiovascular disease is the main cause of premature mortality and two to six time's greater morbidity of T2D patients than of non-diabetic people. There are several mechanisms which could play a role in the pathogenesis of vascular complications and most of them are triggered by hyperglycemia and hyperglycemia-induced oxidative stress (Qasim et al., 2008).

However, during the past few years a lot of attention has been paid to the potential role of adipose

tissue in the development of vascular complications of diabetes. As obesity is considered to be a major risk factor for atherosclerosis, understanding of the underlying mechanisms leading to obesity and linking obesity with atherogenesis is necessary, for the development of therapeutic strategies against atherosclerosis. The pathophysiology of CVD linked to obesity is an area of intensive research (Marinou et al., 2009). The relation between obesity and CVD is indeed complicated. Some investigators suggest that the connection is indirect and dependent on the increased prevalence of diabetes, hypertension and dyslipidemia, whereas others have demonstrated an independent association between obesity (and especially abdominal obesity) and CVD risk (Frayn 2005). The relationship between obesity and CVD appears to develop at a relatively young age. Obesity in young men, aged 15 to 34 years, is associated with accelerated coronary atherosclerosis (McGill et al. 2002).

Excessive adiposity is the most important risk factor in the development of insulin resistance and type 2 diabetes mellitus (Bloomgarden.,2002). The pathophysiology linking obesity to type 2 diabetes is not completely understood, but adipokines are thought to be involved (Jazet et al., 2003). Adipose tissue produces several proteins (adipocytokines) such as leptin, adiponectin, resistin, tumor-necrosis factor-a, and interleukin (IL)-6, that modulate insulin sensitivity and appear to play an important role in the pathogenesis of insulin resistance, diabetes, dyslipidemia, inflammation, and atherosclerosis (Trujillo & Scherer , 2005, Arita et al., 1999 Kershaw and Flier 2004; Arner ,2005; Fantuzzi .,2005; Hotamisligil and Spiegelman 1994; Hotamisligil et al., 1993, Hotamisligil et al.,1994). However, the mechanisms by which fat tissue induces insulin resistance and the role of adipocytokines in the pathogenesis of type 2 diabetes mellitus have not been well established.

Adipokines are fat-secreted biomolecules with diverse signaling effects that modulate insulin resistance, hepatic lipoprotein production, and vascular inflammation . Two in particular, Adiponectin and leptin, are almost exclusively fat derived and have antithetic actions in insulin resistance and in vascular signaling. Because of these properties, Adiponectin and leptin have been proposed as biomarkers of adipose function that may add value in predicting cardiovascular disease (CVD) risk and provide targets for therapeutic interventions (Qasim et al., 2008).

Levels of Adiponectin, an insulin-sensitizing hormone with anti-inflammatory properties, are reduced in obesity, type 2 diabetes, and coronary artery disease (CAD) compared with controls (Behre., 2007) Indeed, several (Schulze et al., 2005, Pischon et al., 2004]) but not all (Lawlor et al., 2005, Lindsay et al., 2005) epidemiologic studies suggest that reduced plasma Adiponectin levels are independent predictors of

Leptin, on the other hand, is a pleiotropic adipokine that modulates innate immune functions and vascular signaling in addition to its central role in regulation of appetite and energy expenditure (Hamann & Matthae, 1996) In contrast to adiponectin, leptin levels directly correlate with insulin resistance, obesity (Chu et al.,2000) and several CVD risk factors (Haynes., 2005) Leptin levels have been associated with CVD beyond body mass index (BMI) in some studies (Reilly et al.,2004) Several large population based studies have suggested strong positive associations between leptin and insulin resistance and its components including hyperlipidemia and hypertension, independent of body mass index (Koerner et al.,2005).

The current study was designed to investigate the endogenous mechanism by which obesity may increase the risk of CVD by examining whether fasting Adiponectin ,leptin or insulin mediate the association of obesity and type2 diabetes, and thrombogenic cardiovascular risk factors in a national population-based cohort of Egyptian adults. A second purpose was to determine whether Adiponectin, leptin and insulin are associated with these CVD risk factors, independent of obesity and type2 diabetes.

Patients and Methods

Patients

This study included 81 subjects, 29 patients suffering from type 2 diabetes selected from National Institute of Diabetes and Endocrinology, 52 patients suffering from type 2 diabetes together with coronary artery disease (CAD) and another group with CAD without diabetes were selected from the National Institute of Heart Disease, they were classified according to their body mass index (BMI) into obese and non-obese groups, obesity was defined according to the WHO criterion (BMI>30kg/m2), also 25 healthy volunteers were considered as controls.

Type 2 diabetes mellitus was diagnosed based on the criteria of World Health Organization criteria (Alberti and Zimmet, 1998). The duration of diabetes was 9.1+ 0.7 years (mean+ SD). No subject had clinical or laboratory signs of acute infection and none had a history or presence of clinically evident cardiovascular disease. They do not receive insulin therapy.

The CAD inclusion criteria were: angiographic evidence with >50% occlusion of ≥1 major coronary arteries, old myocardial infarction, or angina pectoris, but any possible non-Atherogenesis occlusions such as osteal stenosis and spasm were excluded. Electrocardiogram (ECG)) abnormality was

defined when the interpretation was of a trial fibrillation, a major ST-T segment change, left ventricular hypertrophy or a ventricular conduction defect.

All subjects provided informed consent and the study protocol was approved by the Ethics Committee of the Institutional Review Board of National Institute of Diabetes & Endocrinology and National Heart Institute.

All patients were classified according to their body mass index (BMI) into obese and non-obese groups, obesity was defined according to the WHO criterion (BMI>30kg/m2).

- G1: included 10 non-obese suffering from type II diabetes mellitus patients without history of cardiovascular complications (T2DM)
- G2: included 19 non-obese patients suffer from Coronary Artery Disease without suffering from type II diabetes mellitus (CAD)
- G3: included 10 non-obese patients suffer from T2DM+CAD
- G4 included 19 obese suffering from type II diabetes mellitus patients without history of cardiovascular complications
- G5: included 10 non-obese patients suffer from CAD without suffering from type II diabetes mellitus
- G6: included 13 obese patients suffer from T2DM+CAD

Beside reference group included (25) healthy as controls. Criteria for normal controls included (1) absence of history of CHD. (2) Absence of hypertension, diabetes mellitus, or impaired renal function, and (3) Normal ECG and chest x-ray.

Anthropometric assessments

Body mass index:

Body mass index calculated as the body weight divided by the square of the height (kg/m2) was used as a marker of obesity. Weight and height were measured on the third or fourth day after admission while the subjects were fasting and wearing only their undergarments. Patients were designated as obese where BMI exceeded 30 kg/m2 and were considered non-obese where BMI was below 30 kg/m2.

Blood samples and Laboratory assessments:

Venous blood samples were obtained from all the patients on admission to the hospital. Venous blood specimens were collected without EDTA-treated and plain tubes after a 12-h fast. The tubes were immediately placed on ice until they arrived at the laboratory room (within 1–3h) and were stored at −70 °C until analysis. The CVs of the biomarker measurements were all <6%.

For glucose tests, blood was collected in fluorinated tubes and plasma was immediately separated and kept refrigerated at 4° C for up to 48 hours. Plasma glucose was determined by the glucose-oxidase method.

Assessment of insulin resistance

Fasting plasma glucose (FPG) was measured using an enzymatic colorimetric method with glucose oxidase. Insulin was measured by micro particle enzyme assay (Abbott, Chicago, IL). .Homeostasis model assessment (HOMA) or (HOMAIR) was used to detect the degree of insulin resistance (Matthews et al., 1985). In each subject, the degree of insulin resistance can be assessed from the fasting glucose and insulin concentrations by the formula: resistance (HOMA-IR.)

HOMA IR (%) = fasting blood glucose (mg/dL)/18 × fasting insulin (μU/mL)/22.5.

Measurement of serum Adiponectin and Leptin

Serum samples for measurement of Adiponectin and leptin were stored at - 80°C until subsequent analysis. The quantitative determination of human Leptin was conducted in serum by solid phase ELISA techniques, using commercially available kit, purchased from R&D Systems Inc., (Minneapolis, USA). Adiponectin was determined by ELISA (Human Adiponectin ELISA Kit, Linco Research, Inc., St. Charles, MO; intra-assay coefficient of variation, 1.0–7.4%; intra-assay coefficient of variation, 2.4–8.4%; sensitivity, 0.5 ng/ml).

Statistical analyses

Statistical analyses were conducted using SPSS for Windows, Version 11.0 (SPSS, Chicago, IL). Data are expressed as means ± standard deviations

Significant differences between groups were evaluated using ANOVA with post hoc testing. Possible associations between variables were tested using Pearson's correlation .Differences were considered statistically significant at $p < 0.05$.

Results

Table (1a &b) and figure (1&2) summarizes data and Multiple Comparisons (Post Hoc Tests) among the different studied non-obese groups.

-Serum Adiponectin:

In non obese groups

The results revealed that the mean values ± S.E.M of Adiponectin in controls (C) and non obese patients groups T2D (G1), CAD (G2),and T2D+CAD (G3)] were 15.43± 0.57, 10.86 ±0.45, 11.29±0.4,and 11.158±0.68 ng/ml respectively with ranges of 10.8-

20.6, 8.14-12.7, 7.86-14.62,and 7.7-14.38 ng/ml (Table 1). The result showed that the total serum Adiponectin levels were lower than the upper limit in 10 cases (100%), 19 cases (100%) and 10 cases (100%).in group (G1, (G2) and (G3) respectively. The statistical analysis revealed highly significant decrease in non obese groups (G1, G2 and G3) as compared to controls (P<0.001).However, there was no statistical variation between non Obese groups when compared to each others (Table 2a).

In obese groups

The results revealed that the mean values ± S.E.M of Adiponectin in controls (C), obese patient groups [T2D (G4), CAD (G5), and T2D+CAD (G6)] were15.43± 0.57, 9.99 ±0.71, 11.69±0.67,and 9.9±0.4. ng/ml with ranges of 10.8-20.6, 5.18-15.91 , 8.3-14.4, and 7.9-12 ng/ml respectively (Table 1a)

The result showed that the total serum Adiponectin levels were lower than the upper limit in 19 cases (100%) 10 cases (100%) and13 cases (100%) in group G4, G5 and G6. The statistical analysis revealed high significant decrease in obese groups (G4, G5, and G6) when compared to controls (P<0.001).However, there were no statistical variations between obese groups when compared to each others.

-Serum Leptin:

In non Obese

The results revealed that the mean values ± S.E.M of Leptin in controls (C),T2D (G1), CAD (G2),and T2D+CAD (G3) were 3.87± 0.19, 5.66±.0.29, 5.92±.0.45 ,and 6.645±0.57 ng/ml with ranges of 2.17-5.6, 4.1-6.9, 3.4-9.88,and 4.18-9.22 ng/ml. respectively (Table 1a)

The result showed that serum Leptin levels were higher than the upper limit in 5 cases (50%), 10 cases (53%) and 6 cases (32%) in group G1, G2 and G3 respectively.

The statistical analysis revealed highly significant increase in non Obese groups (G1, G2, and G3) as compared to controls (P<0.001).However, there was no statistical variation between obese and non obese.

In Obese

The results revealed that the mean values ± S.E.M of Leptin in controls (C), obeseT2D (G4), obese CAD (G5), and Obese T2D+CAD (G6) were, 3.87±0.19, 5.05±.0.41, 6.56±0.99 and 7.8±0.40 ng/ml respectively with ranges of 2.17-5.6, 2.43-9, 2.36-11.84, and 5.2-10.04 ng/ml (Table 1a). Serum Leptin levels were higher than the upper limit in 11 cases (85%) in group (G4), 5 cases (50%) in group (G5), 7 cases (36%) in group (G6). The statistical analysis revealed high significant increase in obese groups (G4, G5, and G6) as compared to controls (P<0.001).Moreover, there was statistical increase between (G4) and (G5) groups when compared to each others, but there was no significant variation between (G5) and (G6) groups when compared to each others (P>0.05) (Tablevariation between non Obese groups when compared to each others (Table 2a).

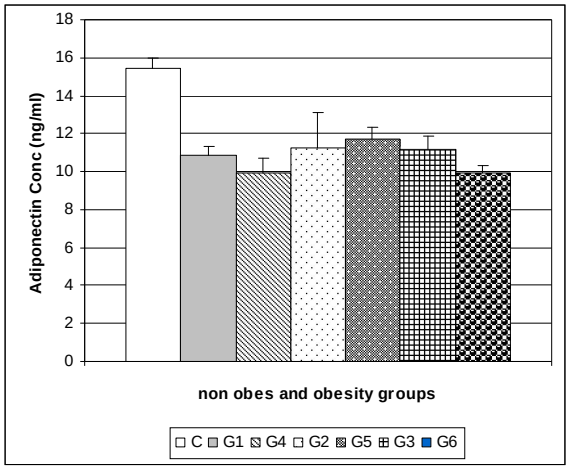


Fig. 1. Adiponectin Conc. (ng/ml) in Different Studied Groups (non obese and obese groups).
C: Controls; G1: T2DM (non obese); G4: T2DM (obese); G2: CAD (non obese); G5: CAD (obese); G3: T2DM+CAD (non obese); G6: T2DM+CAD (obese)

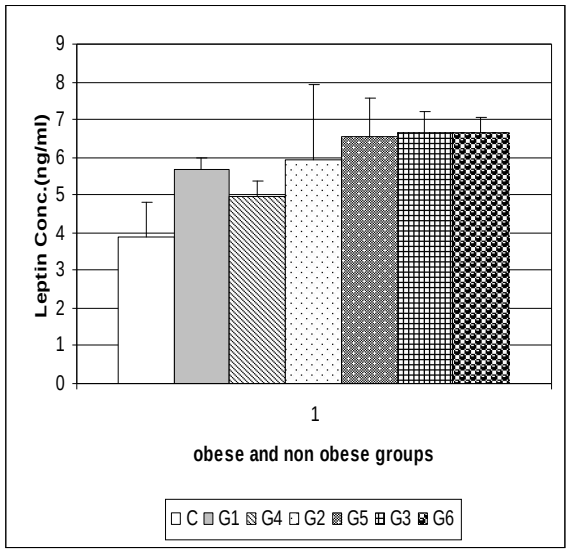


Fig. 2. Leptin Conc. (ng/ml) in Different Studied Groups (non obese and obese groups).
C: Controls; G1: T2DM (non obese); G4: T2DM (obese); G2: CAD (non obese); G5: CAD (obese); G3: T2DM+CAD (non obese); G6: T2DM+CAD (obese)

Table (1a). Collective Data for Serum Adipokines (Adiponectin, Leptin) in non Obese, obese and Healthy Control Groups

Parameters / Groups		**Controls (C)**	**T2DM**		**CAD**		**T2DM +CAD**	
			Non-obese (G1)	Obese (G4)	Non-obese (G2)	Obese (G5)	Non-obese (G3	Obese (G6)
BMI	N	25	10	19	19	10	10	13
	Range	21.70- 25.10	20.00 -29.50	5.18-15.91	22.00-30.00	31.00-36.00	25.00-30.00	31.51-44.00
	Mean	23.0720	26.0500	9.9579	26.3842	32.6000	27.9330	36.0438
	S.D.	1.04184	2.96695	3.25528	2.13027	1.71270	1.87806	3.88264
	S.E.	.20837	.93823	.74681	.48872	.54160	.59389	1.07685
Adiponectin	N	25	10	19	19	10	10	13
	Range	10.80-20.60	8.14-12.71	2.43-9.00	7.86- 14.62	8.30-14.40	7.68-14.38	7.90-12.00
	Mean	15.4300	10.8560	4.9358	11.2858	11.6900	11.1579	9.9231
	S.D.	2.86669	1.41221	1.81995	1.82027	2.11474	2.14920	1.35040
	S.E.	.57334	.44658	.41752	.41760	.66874	.67964	.37453
Leptin	N	25	10	19	19	10	10	13
	Range	2.17-5.60	4.10-6.93	2.43-9.00	3.40-9.88	2.36-11.84	4.18-9.22	5.20-10.04-
	Mean	3.8696	5.6901	4.9358	5.9247	6.5610	6.6450	7.8477
	S.D.	.95263	.92908	1.81995	1.97883	3.13274	1.79425	1.52043
	S.E.	.19053	.29380	.41752	.45397	.99066	.56739	.42169

Table (1b). Collective Data for Serum Glucose, Insulin and HOMA-IR in non Obese, obese and Healthy Control Groups

Parameters / Groups		Controls (C)	T2DM		CAD		T2DM +CAD	
			Non-obese (G1)	Obese (G4)	Non-obese (G2)	Obese (G5)	Non-obese (G1)	Obese (G4)
Glucose	N	25	10	19	19	10	10	13
	Range	75.00-105.00	123.00-311.00	110.00-357.00	82.00-127.00	75.00-114.78	123.00-228.00	141.25-265.00
	Mean	86.2000	198.6000	230.3684	99.6316	93.1780	169.5000	184.8715
	S.D.	9.21954	51.84421	67.55344	12.20751	12.49912	33.67904	40.68162
	S.E.	1.84391	16.39458	15.49782	2.80060	3.95257	10.65025	11.28305
Insulin	N	25	10	19	19	10	10	13
	Range	3.60-8.90	6.11-19.21	2.70- 10.90	1.09-17.66	4.30-55.31	5.24-17.72	6.42-27.21
	Mean	5.4904	10.1360	5.9842	7.8221	11.6450	11.3830	16.5623
	S.D.	1.48685	4.22863	2.35118	4.81128	15.47841	3.72399	5.92084
	S.E.	.29737	1.33721	.53940	1.10378	4.89470	1.17763	1.64214
HOMA-IR	N	25	10	19	19	10	10	13
	Range	.70-2.20	2.80-9.60	2.70-10.90	.30-4.10	.80-11.50	2.30	4.00
	Mean	1.1840	4.8500	5.9842	1.8632	2.5900	4.7800	7.4923
	S.D.	.41501	2.18187	2.35118	1.12852	3.19668	1.94867	3.43765
	S.E.	.08300	.68997	.53940	.25890	1.01088	.61623	.95343

Table 2 (a): Multiple Comparisons (Post Hoc Tests) Between Age, BMI, Adiponectin and Leptin in non obese Patients and healthy control Groups

Dependent Variable	(I) DIAGNOSIS	(J) DIAGNOSIS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
BMI	C	G1	**-2.9780***	.71436	.000	-4.4069	-1.5491
		G2	**-3.3122***	.58108	.000	-4.4745	-2.1499
		G3	**-4.8610***	.71436	.000	-6.2899	-3.4321
	G1	C	**2.9780***	.71436	.000	1.5491	4.4069
		G2	-.3342	.74589	.656	-1.8262	1.1578
		G3	**-1.8830***	.85382	.031	-3.5909	-.1751
	G2	C	**3.3122***	.58108	.000	2.1499	4.4745
		G1	.3342	.74589	.656	-1.1578	1.8262
		G3	**-1.5488***	.74589	.042	-3.0408	-.0568
	G3	C	**4.8610***	.71436	.000	3.4321	6.2899

		G1	**1.8830***	.85382	**.031**	.1751	3.5909
		G2	**1.5488***	.74589	**.042**	.0568	3.0408
ADIPONEC	C	G3	**4.2721***	.92511	**.000**	2.4051	6.1391
		G1	**4.5740***	.92511	**.000**	2.7070	6.4410
		G2	**4.1442***	.69890	**.000**	2.7462	5.5422
	G3	C	**-4.2721***	.92511	**.000**	-6.1391	-2.4051
		G1	.3019	1.10572	.786	-1.9295	2.5333
		G2	-.1279	.89714	.887	-1.9224	1.6667
	G1	C	**-4.5740***	.92511	**.000**	-6.4410	-2.7070
		G2	-.4298	.89714	.634	-2.2243	1.3648
		G3	-.3019	1.10572	.786	-2.5333	1.9295
	G2	C	**2.0551***	.44628	**.000**	1.1624	2.9478
		G2	.2346	.57286	.684	-.9113	1.3805
		G3	-.7203	.57286	.214	-1.8662	.4256
LEPTIN	C	G3	**-2.7754***	.44167	**.000**	-3.6667	-1.8841
		G2	**-2.0551***	.44628	**.000**	-2.9478	-1.1624
		G1	**-1.8205**	.44167	**.000**	-2.7118	-.9292
	G3	C1	**2.7754***	.44167	**.000**	1.8841	3.6667
		G1	.9549	.52790	.078	-.1104	2.0202
		G2	.7203	.57286	.214	-.4256	1.8662
	G1	C	**1.8205***	.44167	**.000**	.9292	2.7118
		G3	-.9549	.52790	.078	-2.0202	.1104
		G3	-.2346	.57286	.684	-1.3805	.9113
	G2	C	**2.0551***	.44628	**.000**	1.1624	2.9478
		G1	.2346	.57286	.684	-.9113	1.3805
		G3	-.7203	.57286	.214	-1.8662	.4256

Based on observed means.

***The mean difference is significant at the .05 level.

N.B.: C=controls G1= T2DM

G2 = CAD G3= CAD+T2DM

Table 2 (b): Multiple Comparisons (Post Hoc Tests) Between Glucose, Insulin and HOMA.IR in non obese Patients and healthy control Groups

Dependent Variable	(I) DIAGNOSIS	(J) DIAGNOSI	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
GLUCOSE	C	G1	**-112.400***	9.55422	**.000**	-131.5113	-93.2887
		G2	-13.4316	7.77160	.089	-28.9771	2.1139
		G3	**-83.3000***	9.55422	**.000**	-102.4113	-64.1887
	G1	C	**112.4000***	9.55422	**.000**	93.2887	131.5113
		G2	**98.9684***	9.97593	**.000**	79.0136	118.9232
		G3	**29.1000***	11.41947	**.013**	6.2576	51.9424
	G2	C	13.4316	7.77160	.089	-2.1139	28.9771
		G1	**-98.9684***	9.97593	**.000**	-118.9232	-79.0136
		G3	**-69.8684***	9.97593	**.000**	-89.8232	-49.9136
	G3	C	**83.3000***	9.55422	**.000**	64.1887	102.4113
		G1	**-29.1000***	11.41947	**.013**	-51.9424	-6.2576
		G2	**69.8684***	9.97593	**.000**	49.9136	89.8232
INSLUIN	C	G1	**-4.6456***	1.32769	**.001**	-7.3014	-1.9898
		G2	**-2.3317***	1.07997	**.035**	-4.4920	-.1714
		G3	**-5.8926***	1.32769	**.000**	-8.5484	-3.2368
	G1	C	**4.6456***	1.32769	**.001**	1.9898	7.3014
		G2	2.3139	1.38630	.100	-.4591	5.0869
		G3	-1.2470	1.58690	.435	-4.4213	1.9273
	G2	C	**2.3317***	1.07997	**.035**	.1714	4.4920
		G1	-2.3139	1.38630	.100	-5.0869	.4591
		G3	**-3.5609***	1.38630	**.013**	-6.3339	-.7879
	G3	C	**5.8926***	1.32769	**.000**	3.2368	8.5484
		G1	1.2470	1.58690	.435	-1.9273	4.4213
		G2	**3.5609***	1.38630	**.013**	.7879	6.3339
HOMA	C	G3	**-3.5960**	.52011	**.000**	-4.6456	-2.5464
		G1	**-3.6660**	.52011	**.000**	-4.7156	-2.6164
		G2	-.6792	.40085	.095	-1.4810	.1227
	G3	C	**3.5960**	.52011	**.000**	2.5464	4.6456
		G1	-.0700	.62165	.911	-1.3245	1.1845
		G2	**2.9168***	.51455	**.000**	1.8876	3.9461

G1	C	**3.6660**	.52011	**.000**	2.6164	4.7156
	G2	**2.9868***	.51455	**.000**	1.9576	4.0161
	G3	.0700	.62165	.911	-1.1845	1.3245
G2	C	.6792	.40085	.095	-.1227	1.4810
	G1	**-2.9868***	.51455	**.000**	-4.0161	-1.9576
	G3	**-2.9168***	.51455	**.000**	-3.9461	-1.8876

Based on observed means.

***The mean difference is significant at the .05 level.

N.B.: C=controls G1= T2DM

G2 = CAD G3= CAD+T2DM

Table 3 (a). Univariate analysis of correlation between Adipokines (Adiponectin & Leptin) in T2DM groups and other variables (all results are two-tailed)

Variable	***Adiponectin***				***Leptin***				***HOMA-IR***			
	Non obese		**obese**		**Non obese**		**obese**		**Non obese**		**obese**	
	R	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**
BM	-.491	.149	.373	.116	.470	.171	.269	.265	**.698***	**.025**	.427	.069
Adiponectin	1	.	1	.	.228	.526	**.523***	**.021**	-.472	.168	.109	.658
Leptin	.228	.526	**.523***	**.021**	1	.	1	.	-.087	.812	.431	.066
Glucose	-.062	.866	-.004	.988	-.185	.608	.142	.561	.424	.222	.412	.080
Insulin	-.349	.323	.102	.676	.081	.823	.218	.370	**.786****	**.007**	**.594****	.007
HOMA-IR	-.472	.168	.109	.658	-.087	812	.431	.066	1	.	1	.

Table 3 (b). Univariate analysis of correlation between Adipokines (Adiponectin & Leptin) in CAD groups and other variables (all results are two-tailed)

Variable	***Adiponectin***				***Leptin***				***HOMA-IR***			
	Non obese		**obese**		**Non obese**		**obese**		**Non obese**		**obese**	
	R	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**
BM	-.328	.170	.014	.969	.038	.876	-.121	.738	.215	.377	-.200	.580
Adiponectin	1	.	1	.	.443	.058	.378	.282	.224	.356	.539	.108
Leptin	.443	.058	.378	.282	1	.	1	.	.418	.075	.246	.493
Glucose	-.250	.303			**-.604****	**.006**	-.206	.569	-.326	.173	.780	-.102
Insulin	.247	.308	.514	.128	**.499***	**.030**	.272	.447	**.985****	**.000**	.996	.000
HOMA-IR	.224	.356	.539	.108	.418	. .075	.246	.493	1	.	1	.

Table 3 (C). Univariate analysis of correlation between Adipokines (Adiponectin & Leptin) in T2DM+CAD groups and other variables (all results are two-tailed)

Variable	***Adiponectin***				***Leptin***				***HOMA-IR***			
	Non obese		**obese**		**Non obese**		**obese**		**Non obese**		**obese**	
	R	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**
BM	.533	.113	-.094	.759	-.222	.538	.113	.713	**-.716***	**.020**	-.202	.508
Adiponectin	1	.	1	.	.376	.285	**.660***	**.020**	-.288	.420	-.202	.509
Leptin	.376	.285	**.660***	**.020**	1	.	1	3	.517	.126	-.046	.882
Glucose	.165	.649	.253	.404	.565	.089	.273	.367	.524	.120	.367	.218
Insulin	-.340	.336	.265	.509	.382	.276	-.197	.520	**0.902****	**.000**	**0.868***	**000**
HOMA-IR	-.288	.420	-.202	.509	.517	.126	-.046	.882	1	.	1	.

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

HOMA-IR Ratio:

In non obese

The results revealed that the mean values ± S.E.M of HOMA-IR Ratio in controls (C),T2D (G1), CAD (G2),and T2D+CAD (G3) were1.8±0.082, 4.85±0.69, 1.86±0.26,and 4.78±0.62 with ranges of, 0.7-2.2, 6.1-19.21, 0.3-4.1,and 2.3-8.2 respectively (Table 1b). The result showed that the HOMA-IR Ratio was higher than the upper limit in 10 cases (100%) ,6 cases (32%) and 6 cases (60%) in group G1,G2and G3 respectively. The statistical analysis revealed highly significant increase in non obese groups (G1,G2and G3) as compared to both controls and each other (P<0.001).(Table 2b).

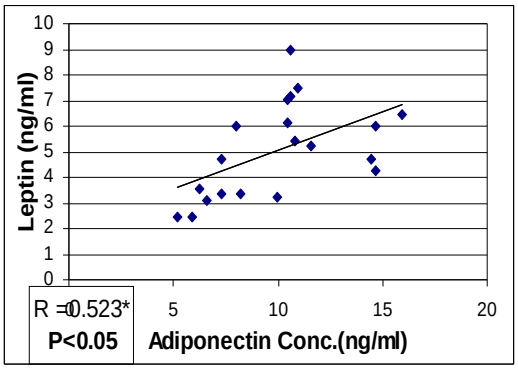
In Obese

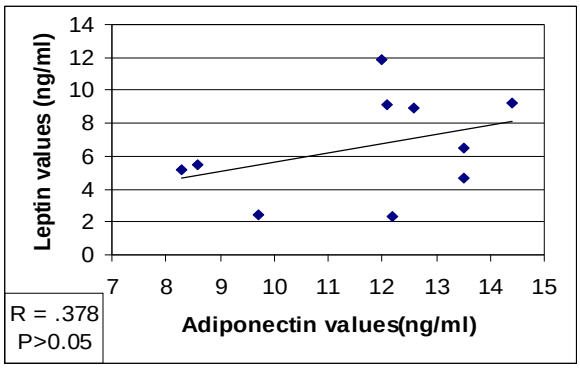
The results revealed that the mean values ± S.E.M of HOMA-IR Ratio in controls (C),T2D (G4), CAD (G5),and T2D+CAD (G6) were1.18±0.082, 5.96±0.51, 2.59±1.01,and 7.5±1.0 respectively with ranges of, 0.7-2.2 , 2.7-10.9,0.8-11.5,and 4-16.5. The result showed that the HOMA-IR Ratio was higher than the upper limit in 19 cases (100%) in group (G4),2 cases (20%) in group (G5),and 13 cases (100%) in group (G6).

The statistical analysis revealed highly significant increase in the two obese groups only (G4, and G6) as compared to controls (P<0.001). While there was no significant variation in (G5) when compared to (C) (P>0.05).Moreover, there was a significant increase in all obese groups when compared to each others (P<0.05). The current study showed also a significant correlation between serum adiponectin and leptin in obese DM patients (Figure2).

Relationship between adipocytokines and other parameters

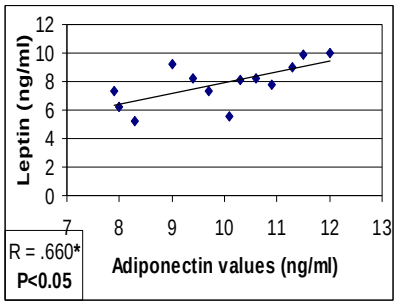
Data on the relationship between Adiponectin and the other variables in non obese and obese patient groups are shown in Tables (2a, b, c). Serum Adiponectin levels were poor associated with Leptin CAD (obese) {r=0.378, P=0.282) (Table (3a, C) & Fig 3) Whereas Adiponectin were associated with Leptin in both of obese T2DM without and CAD (r=0.0.523; P=0.0021; r=0.660; P=0.020) respectively (Table 3b & Fig 3). Leptin Levels were good associated with Insulin in CAD (non obese) (r=0.499, P<0.03).





(G4)T2DM (Obese) **(G5) CAD (obese)**

Fig 3a. Correlation between Adiponectin and Leptin in obese T2DM and CAD Groups.



(G6) T2DM+CAD (obese)

Fig 3b. Correlation between Adiponectin and Leptin in obese T2DM+ CAD Group. Correlation is significant at the 0.05 level.

Discussion

The current study demonstrated that serum leptin concentration was significantly higher in obese DM + CAD patients than obese patients with CAD alone. These findings suggested that the hyperinsulinaemia that accompanies obesity and diabetes in our DM+CAD patients probably results in increased ob gene expression and higher plasma leptin concentration This agreed with previous studies that showed that leptin is part of the adipoinsular axis, as insulin stimulates leptin production in adipocytes and leptin interacts with the pancreatic B-cell through various effects on insulin secretion (Bullo et al. 2002) It has been shown in animal studies , insulin increases the expression of the ob gene and results in increased leptin concentration, while relative or absolute deficiency of insulin results in reduced ob gene expression(Friedman and Halaas 1998)

The increased leptin levels in obese DM+CAD, obese DM, and obese CAD patients in current study might represent an integrated marker of adiposity, insulin resistance, and vascular dysfunction in Egyptian patients This integrity agrees with previous studies by (Wallace et al. 2001) who postulated that hyperleptinimeia has been recognized as a mediator between obesity and cardiovascular disease.The effect of obesity and high leptin levels on vascular system likely due to several various factors such as increasd sympathetic activity,enhanced platelet aggregation, increased oxidative stress, and cardiac hypertrophy(Konstantinides et al. 2001; Bodary et al. 2002; Paolisso et al. 1999) Besides, increased adipose tissue in obesity requires an increased vascular bed to maintain its baseline circulation (Bouloumie et al. 1998; Sierra-Honigmann et al. 1998) Thus this adaptation may, conversely, promote arteriosclerosis over long periods of time.

Higher leptin concentrations were shown to be associated with an impaired arterial distensibility(Singhal et al. 2002), and patients with a restenosis after coronary stenting had higher leptin levels than those without a restenosis(Piatti et al. 2003) Furthermore, the direct influence of leptin on the vascular biology is supported by the *ob/ob* mice, which lacks leptin and consequently becomes hyperphagia and obesity but is nevertheless resistant to atherosclerosis(Schafer et al. 2004) However,the administration of leptin removes this protective effect against atherosclerosis.The atherosclerosis risk in heterozygote and the control mouse,which suggest a dose-response relation between the leptin levels and the atherosclerotic process(Nishina et al. 1994)

Our findings also showed increased levels of insulin in studied obese patients.The increase in leptin levels in these groups did not show significant correlation with insulin resistance This agreed with evidence that plasma leptin may vary much more as a function of the circulating insulin concentrations than of the degree of the insulin resistance itself (Mohamed-Ali et al. 1997; Lonnqvist et al. 1995) The current study also showed a significant decrease in serum adiponectin levels in obese and non obese CAD, and DM+CAD patients compared to controls, consistent with our findings, previous studies that showed a decreased levels of adiponectin in a variety of insulin-resistant states, including obesity , diabetes, and cardiovascular diseases(Hotta et al. 2000; Weyer et al. 2001; Kumada et al. 2003; Trujillo and Scherer 2005) .

There were some possibilities explained the decrease in serum levels of adiponectin in different studied obese and non obese groups. One of them is elevated insulin levels in diabetic subjects may have been responsible for the decreased adiponectin concentrations as insulin regulates the secretion of various proteins from adipose tissue(Yu et al. 2002; Mohlig et al. 2002). There is evidence that insulin may have direct effect on adiponectin gene expression and adiponectin concentrations in vitro (Fasshauer et al. 2002; Halleux et al. 2001; Motoshima et al. 2002). Another possibility was that accumulation of adiponectin in atherosclerotic vascular walls may accelerate its half-life in plasma, resulting in the reduction of the plasma concentration of adiponectin in subjects with CAD (Hotta et al. 2000) . The current study showed significant correlation between serum adiponectin and leptin in obese DM patients, similar results were obtained by Satoh et al. 2004; Kotani et al. 2005).

Conclusion

The coexistence of a correlation between serum leptin and Adiponectin levels in addition to increase of serum leptin and decrease serum Adiponectin levels in obese DM patients in the current study, support that hypothesis of their susceptibility to atherosclerosis. Although the small sample size does

not enable us to make a definitive conclusion, our study revealed that there were significant differences in plasma levels of Adiponectin between CAD patients with and without DM. DM has an additional effect on CAD, that of decreasing plasma Adiponectin levels. We speculate that people who have very low plasma Adiponectin levels may be at increased risk of developing both CAD and DM.

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Corresponding Author:
Dr. Nervana Samy
Department of Biochemistry
Genetic Engineering & Biotechnology Devision.
National Research Centre
Dokki, Giza, Egypt
e.mail: Nervana91@hotmail.com

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