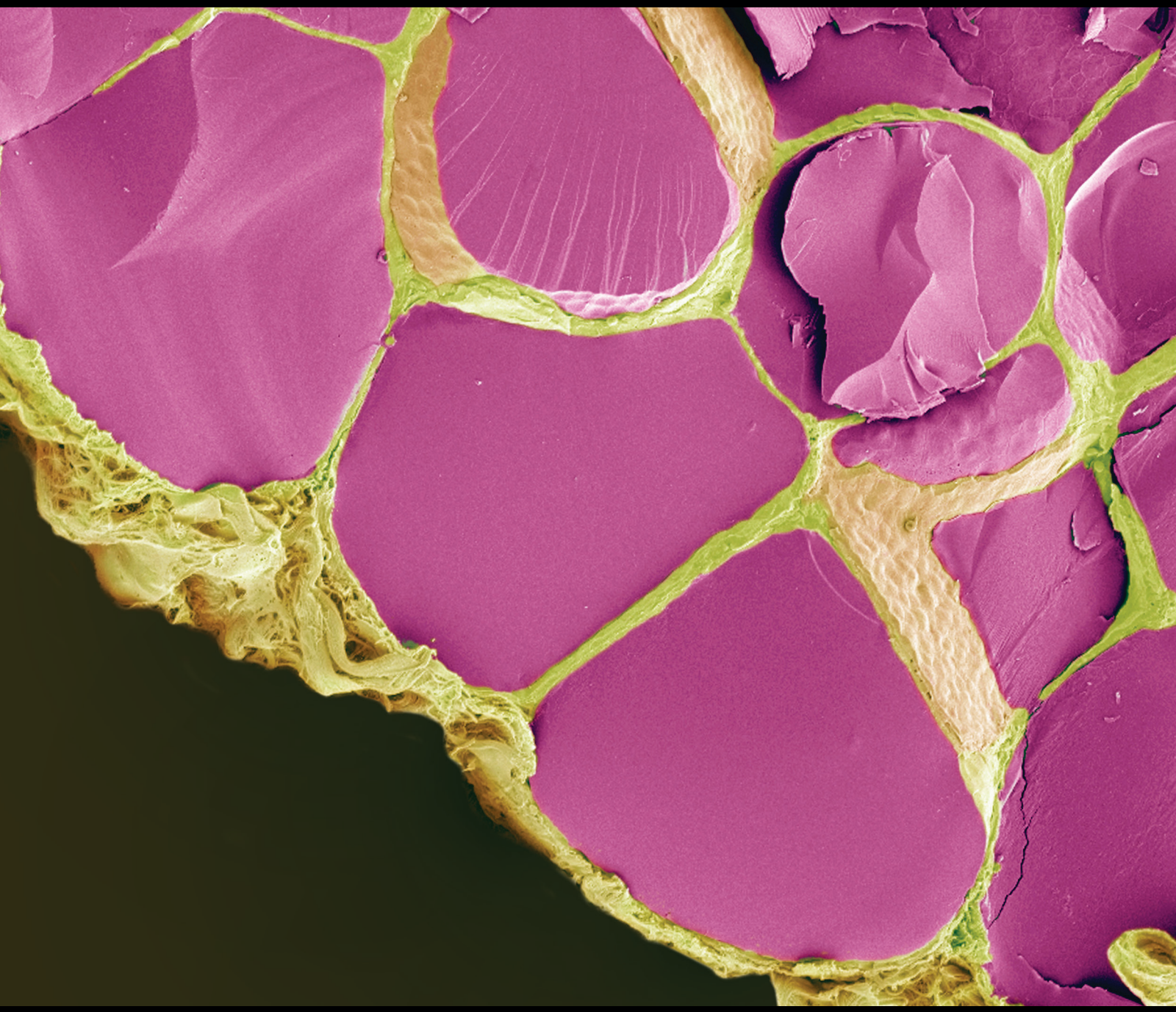
International Journal of Endocrinology

Type 2 Diabetes and Cardiovascular Risk in Women

Guest Editors: Giuseppina T. Russo, Giovannella Baggio, Maria Chiara Rossi, and Alexandra Kautzky-Willer



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Editorial

Type 2 Diabetes and Cardiovascular Risk in Women

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Cardiovascular diseases (CVD) are the leading cause of death, also in diabetic women. Since 1998, when Haffner et al. [1] stated that subjects with type 2 diabetes mellitus (T2DM) had a CVD risk "equivalent" to previous myocardial infarction, a large number of studies have shown that this relative risk for CVD due to diabetes is greater in women than in men [2].

CVD in diabetic subjects is not entirely related to chronic hyperglycaemia and a number of other factors such as dyslipidemia, hypertension, hormonal, genetic, and environmental factors, as well as low-grade systemic inflammation and endothelial damage, lifestyle behaviours, adherence to therapies, and/or psychosocial factors may contribute to the worst outcomes observed in diabetic women. Notably, it is increasingly recognized that many of these factors show gender differences in their prevalence and/or association with CVD events, and this aspect should be specifically targeted when aiming at primary or secondary CVD prevention in diabetic subjects.

In this special issue, we looked at CVD in women with diabetes from different perspectives, giving a great contribution to this topic, in terms of mortality, management of risk factors, and therapies.

Two papers of this special issue looked at sex differences in CVD mortality associated with diabetes. One conducted on a large population-based sample from Italy demonstrated an excess of mortality in diabetic subjects as compared to nondiabetic ones and a greater impact of diabetes in females than in males for mortality for all causes, for CVD, and for myocardial infarction and renal causes. In the other study, G. Luo et al. showed in a retrospective analysis that fasting plasma glucose was an independent predictor of in-hospital mortality for nondiabetic female patients.

Gender-specific prevalence and management of major and emerging CVD risk factors in different populations were also the main topic of several papers of this special issue.

The paper by S. Chen et al., with a very interesting experimental protocol, clarified the relationships of albuminuria, a well-recognized CVD risk factor, with circulating levels of angiopoietin-1 (Ang-1), Ang-2, and vascular endothelial growth factor (VEGF) in serum and urine.

Potential gender differences in the distribution and control of major CVD risk factors were investigated in another three very large high-risk populations. Thus, in the eControl Study, a study on 286,791 patients with T2DM in Catalonia, Spain, J. Franch-Nadal et al. found that cardiometabolic control was worse in subjects with prior CVD; but control of several risk factors showed gender differences, favouring women with prior CVD only for smoking and BP, whereas LDL-cholesterol (LDL-C) levels were remarkably uncontrolled in women both with and without CVD.

The results of an overall bad control of LDL-C in women were also demonstrated in a very large Italian diabetic outpatient population from the Annals Study Initiative. This study, conducted on 415.294 patients (45.3% women) from

236 diabetes outpatient centers in Italy, demonstrated that LDL-C management was worst in women with T2DM, who were monitored and reached targets less frequently than men and, similar to men, did not receive medications despite high LDL-C levels. These gender discrepancies increased with age and diabetes duration, exposing older women to higher CHD risk.

In accordance with the previously cited studies, also the BARI 2D Trial, in a very high-risk population of T2DM subjects with established coronary artery disease, found that although women were as aggressively treated with drugs as men, they less frequently met targets for HbA1c and LDL-C, suggesting potential sex differences in response to drug therapies used to treat diabetes, hypertension, and hyperlipidemia.

Beyond LDL-C, different papers of this special issue focused on atherogenic dyslipidemia, that is, the presence of low HDL-cholesterol (HDL-C), and high triglycerides, which may have an impact on CVD especially in diabetic women [3, 4]. K. Song et al. demonstrated, in an *in vitro* study on primary hepatocytes of SR-BI knockout (SR-BI−/−) mice, that ATPase-B1 is involved in HDL endocytosis and it may be a potential target for regulating HDL metabolism.

Diabetic women have been demonstrated to have a less atheroprotective HDL subpopulation profile when compared to nondiabetic ones [5]. Another study of the special issue examining HDL subpopulations distribution in that same population of CHD-free diabetic and control women showed an inverse relationship between markers of systemic inflammation and the more atheroprotective large α-1, α-2, and preβ-1 HDL subclasses, especially in diabetic women, suggesting that different HDL particles could affect the atherosclerotic process through the modulation of subclinical inflammation. A third paper on atherogenic dyslipidemia by J. He et al. demonstrated gender differences on the discriminatory power of triglyceride (TG) and the triglyceride to high-density lipoprotein-cholesterol ratio (TG/HDL-C) for insulin resistance in normoglycaemic Chinese subjects.

Furthermore, in normoglycaemic women with previous GDM, Y. Winhofer et al. demonstrated that despite the diagnosis of normal glucose tolerance after delivery, prior GDM women are characterized by persisting subtle glucose abnormalities and insulin resistance, decreased adiponectin, and increased CRP concentrations, thus exposing them to an increased CVD risk. Thus women with a history of GDM should be regarded as high-risk group for development of overt diabetes and CVD and special attention should be given to preventive measures among these females.

Another two articles of this special issue also examined the role of estrogen replacement therapy (ERT) or flavonoids on CVD risk factors. Thus, M. Boukhris et al. reviewed the controversial role of ERT on CHD in postmenopausal type 2 diabetic women, trying to define the place of ERT on CHD prevention, whereas R. D'Anna et al. investigated the effects of a 6-month treatment of polyphenols, myo-inositol, and soy isoflavones on several CVD risk factors in postmenopausal women with metabolic syndrome.

Finally, in a very interesting review, E. Satta et al. explored other aspects of the management of high-risk metabolic women, focusing on sexual dysfunctions in women with diabetic kidney disease and exploring the role of metabolic and hormonal variables and frequently used drugs on this important aspect impacting their quality of life.

Even though the papers published in this special issue are profoundly different in topic and study methodologies, they overall underline the importance of sex differences when examining CVD risk factors and their association with treatment, outcome, and mortality, especially in T2DM subjects.

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Giovannella Baggio
Maria Chiara Rossi
Alexandra Kautzky-Willer

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Research Article

Sex Differences in Cardiovascular Mortality in Diabetics and Nondiabetic Subjects: A Population-Based Study (Italy)

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The objective of this study is to assess the impact of diabetes on cardiovascular mortality, focusing on sex differences. The inhabitants of Reggio Emilia province on December 31, 2009, aged 20–84 were followed up for three years for mortality. The exposure was determined using Reggio Emilia diabetes register. The age-adjusted death rates were estimated as well as the incidence rate ratios using Poisson regression model. Interaction terms for diabetes and sex were tested by the Wald test. People with diabetes had an excess of mortality, compared with nondiabetic subjects (all cause: IRR = 1.68; 95%CI 1.60–1.78; CVD: IRR = 1.61; 95%CI 1.47–1.76; AMI: IRR = 1.59; 95%CI 1.27–1.99; renal causes: IRR = 1.71; 95%CI 1.22–2.38). The impact of diabetes is greater in females than males for all causes ($P = 0.0321$) and for CVD, IMA, and renal causes. Further studies are needed to investigate whether the difference in cardiovascular risk profile or in the quality of care delivered justifies the higher excess of mortality in females with diabetes compared to males.

1. Introduction

Diabetes is now one of the most common noncommunicable diseases globally. The International Diabetes Federation (IDF) most recent estimates indicate that 8.3% of adults—382 million people—have diabetes. Further, the number of people with the disease is expected to rise beyond 592 million in less than 25 years. Yet with 175 million cases currently undiagnosed, a vast number of people who are unaware that they have diabetes are progressing towards complications [1]. In Italy, the overall prevalence in 2011 was about 5%; that is, 1,383,000 men and 1,556,000 women have diabetes [2].

Diabetes and its complications are major causes of early death in most countries. In Europe, one in 10 deaths in adults can be attributed to diabetes, that is 619,000 in 2013 [1]. Cardiovascular disease (CVD), the first cause of death in many industrialized countries, is responsible for a large part of the excess mortality observed among people with diabetes [3]. Indeed, individuals with diabetes have an increased risk of all-cause mortality and morbidity related to CVD compared with individuals without diabetes [3–9].

Nevertheless, the effect of diabetes on CVD seems to be different for males and females [10–17]. In fact, despite the fact that in many industrialized countries women have lower mortality rates than men, when we look at people with diabetes, the advantage for women is reduced or even absent [18, 19]. Estimates of CVD mortality in men with diabetes have varied from 1 to 3 times the rate in men free of the disease, whereas estimates in women with diabetes have ranged from 2 to 5 times the rate in women without diabetes [20–23]. The variation in relative risk estimates of cardiovascular disease makes it difficult to evaluate the strength of diabetes as a risk factor for either sex.

The objective of this study is therefore to assess the impact of diabetes on cardiovascular mortality, focusing on sex differences.

2. Methods

2.1. Setting and Study Population. This study is a retrospective cohort including the inhabitants of Reggio Emilia province (northern Italy) on December 31, 2009, aged 20–84.

To identify people with diabetes (i.e., exposed group) we used the Reggio Emilia diabetes register (accessed on May 21, 2014). The methods applied to develop our disease register have been described elsewhere [24]. In brief, the register was created by deterministic linkage of six routinely collected data sources through a definite algorithm able to ascertain cases and to distinguish type of diabetes and model of care. The sources are hospital discharge, drug dispensation, HbA1c values from biochemistry laboratory, disease-specific exemption, diabetes outpatient clinics, and mortality databases. Women with gestational diabetes or women receiving treatment for polycystic ovarian syndrome were excluded.

2.2. Follow-Up, Outcome, and Covariates. Cohort was followed up for three years (2010–2012). Vital status (alive or dead) information was retrieved from civil register. The subjects who emigrated were treated as censored at the time of emigration.

The outcome of interest was mortality attributable to all causes (ICD-10 A00-T98), cardiovascular disease (CVD) (ICD-110 I00-I99), acute myocardial infarction (AMI) (ICD-10 I21-I23), diabetes (ICD-10 E10-E14), and renal diseases (ICD-10 N00-N39). The causes of death were ascertained using Reggio Emilia mortality register, which contains all resident deaths by year of death, with cause of death coded using *International Classification of Diseases*, tenth revision (ICD-10). Sex and age were considered covariates in the analysis. As a proxy of disease severity, the subjects with diabetes were classified based on treatment: diet only, oral antidiabetic drugs, or insulin. Subjects who were prescribed both insulin and oral antidiabetic drugs were assigned to "insulin treatment" [25].

2.3. Statistical Methods. Characteristics of the study population are presented as median and proportions and stratified by sex and diabetes status. Person-time at risk was calculated from January 1, 2010, to date of death or date of emigration or December 31, 2012.

We calculated proportional mortality by age and diabetes status for principal groups of cause of death.

Then we estimated age-adjusted death rates (AADR) per 100000 with 95% confidence intervals (95% CI), by sex and diabetes status using Italian population on December 31, 2009, as reference for standardization [26]. At the same time, we calculated incidence rate ratios (IRR) and 95% confidence intervals (95% CI) using multivariate Poisson regression model. The individuals without diabetes were used as the reference group, the age as continuous variable, and the sex as covariate. Interaction terms for diabetes and sex were tested by the Wald test.

Further, we estimated incidence rate ratios (IRR) and 95% confidence intervals (95% CI) and risk difference (per 100000) within age category, for all causes, CVD, and AMI and renal causes, and we graphed the age-specific death rates stratified by sex and diabetes status.

Analyses were performed using the STATA statistical package, version 11.0.

2.4. Ethical Approval. This is an observational study and data were collected retrospectively. The Local Health Authority of Reggio Emilia was responsible for collecting and processing these sets of data. The study was commissioned by the Local Health Authority. The Reggio Emilia diabetes registry was approved by provincial Ethic Committee in July 2014. According to Italian privacy law, no patient or relative's consent is required for large retrospective population-based studies.

3. Results

The study cohort consisted of 407,161 subjects (Table 1), 23,438 of whom were diabetic patients (i.e., exposed group) (5.8% of the population): 13074 males and 10364 females (prevalence 6.5% and 5.0%, resp.). Subjects without diabetes were younger and there was a higher percentage of foreigners. The percentage of lost to follow-up because of move was very low in both groups.

Over the three-year study period, 9,208 (2.3%) individuals died; the proportion of deaths was higher in people with diabetes than the unexposed population (8.7% and 1.9%, resp.). The risk was greater in males than females in both groups.

Finally, among people with diabetes, there were no differences by sex in terms of type of treatment ($P = 0.120$).

The distribution of causes was similar for the two populations (Table 2), with the exception of death for endocrine, nutritional, and metabolic causes (which includes diabetes) (E00-E90), where the percentage was 10.3% for males and 11.4% for females with diabetes, compared to 0.7% and 0.9% for males and females without diabetes, respectively. The pattern of mortality by sex was similar in the two subgroups, except for the digestive and renal causes. In females, the proportion of deaths for CVD was 33.2%, with a slight difference between diabetics and nondiabetics subjects (34.4% and 32.9% resp.); in males the percentage of deaths for CVD causes was lower (28.5%) and similar in the two groups.

Diabetic subjects showed an increased risk of all-cause mortality compared to nondiabetics of dying for all causes (Table 3). The excess of risk was found in all categories of causes analyzed in our study.

The analysis by sex indicated that the excess of risk was more evident in diabetic females than diabetic males compared to their nondiabetic counterparts (IRR 1.77; 95% CI 1.64–1.92; IRR 1.63; 95% CI 1.52–1.73, resp.). The effect modification of sex on the association between diabetes and

Table 1: Characteristics of the study cohort by diabetes status and sex.

Characteristics	No diabetes		Diabetes		Total	
	Males	Females	Males	Females	Males	Females
Population 20–84 years	187886	195837	13074	10364	200960	206201
Foreigners*: *N* (%)	25749 (13.7)	28104 (14.3)	950 (7.3)	862 (8.3)	26699 (13.3)	28966 (14.0)
Age (years): median (IQR)	44 (34–59)	47 (35–62)	66 (57–74)	69 (60–76)	46 (35–61)	48 (36–63)
Dead: *N* (%)	4090 (2.2)	3090 (1.6)	1240 (9.5)	788 (7.6)	5330 (2.6)	3878 (1.9)
Emigrated: *N* (%)	417 (0.2)	319 (0.2)	16 (0.1)	12 (0.1)	433 (0.2)	331 (0.2)
Person-years	558521	583162	37234	29842	595755	613004
Diabetes treatment regimen						
Diet only			3213 (24.6)	2626 (25.3)		
Oral drugs			6760 (51.7)	5219 (50.4)		
Insulin			3101 (23.7)	2519 (24.3)		

*Based on the country of birth.

Table 2: Proportional mortality by diabetes status and sex.

Causes: *N* (%)	No diabetes		Diabetes		Total	
	Males	Females	Males	Females	Males	Females
Infectious and parasitic diseases (A00–B99)	97 (2.4)	72 (2.3)	39 (3.1)	30 (3.8)	136 (2.6)	102 (2.6)
Neoplasms (C00–D48)	1680 (41.1)	1176 (38.1)	426 (34.4)	239 (30.3)	2106 (39.5)	1415 (36.5)
Endocrine, nutritional, and metabolic diseases (E00–E90)	30 (0.7)	29 (0.9)	128 (10.3)	90 (11.4)	158 (3.0)	119 (3.0)
Mental and behavioral disorders (F00–F99)	61 (1.5)	87 (2.8)	13 (1.0)	7 (0.9)	74 (1.4)	94 (2.4)
Diseases of the nervous system (G00–G99)	146 (3.6)	132 (4.3)	19 (1.5)	23 (2.9)	165 (3.1)	155 (4.0)
Diseases of the circulatory system (I00–I99)	1161 (28.4)	1017 (32.9)	357 (28.8)	271 (34.4)	1518 (28.5)	1288 (33.2)
Diseases of the respiratory system (J00–J99)	335 (8.2)	205 (6.6)	102 (8.2)	38 (4.8)	437 (8.2)	243 (6.3)
Diseases of the digestive system (K00–K93)	154 (3.8)	130 (4.2)	67 (5.4)	36 (4.6)	221 (4.1)	166 (4.3)
Renal causes (N00–N99)	87 (2.1)	59 (1.9)	25 (2.0)	21 (2.7)	112 (2.1)	80 (2.1)
Injury, poisoning, and other certain consequences of external causes (S00–T98)	244 (6.6)	110 (3.6)	44 (3.5)	17 (2.2)	288 (5.4)	127 (3.3)
Unknown	46 (1.1)	22 (0.7)	13 (1.0)	5 (0.6)	59 (1.1)	27 (0.7)
Other*	49 (1.2)	51 (1.7)	7 (0.6)	11 (1.4)	56 (1.1)	62 (1.6)
Total (A00–T98)	**4090**	**3090**	**1240**	**788**	**5330**	**3878**

*Others include cases classified in the following chapters: III, Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism (D50–D89) ($N = 20$); VII, Diseases of the eye and adnexa (H00–H59) ($N = 1$); VIII, Diseases of the ear and mastoid process (H60–H95) ($N = 1$); XII, Diseases of the skin and subcutaneous tissue (L00–L99) ($N = 14$); XIII, Diseases of the musculoskeletal system and connective tissue (M00–M99) ($N = 34$); XVII, Congenital malformations, deformations and chromosomal abnormalities (Q00–Q99) ($N = 11$); XVIII, Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified (R00–R99) ($N = 37$).

death was statistically significant (Wald test for interaction, $P = 0.0321$).

Looking at cardiovascular mortality, we observed a similar pattern: an excess of risk in people with diabetes, found in both sexes, greater in females than males (males: IRR 1.56; 95% CI 1.38–1.76; females: IRR 1.69; 95% CI 1.47–1.93; Wald test for interaction, $P = 0.1266$).

Among the CVD causes, we observed that for AMI the excess mortality for females with diabetes was more pronounced (males: IRR 1.48; 95% CI 1.10–1.99; females: 1.81; 95% CI 1.27–2.59; Wald test for interaction, $P = 0.1063$).

In the group of renal causes of death, the excess of mortality in the diabetic population was again more evident in females than males (males: IRR 1.37; 95% CI 0.88–2.14; females: 2.37; 95% CI 1.43–3.91; Wald test for interaction, $P = 0.1466$). This group of causes includes those related to kidney dysfunctions, such as glomerular diseases, renal tubulointerstitial diseases, acute kidney failure, chronic kidney disease,

Table 3: *N* of dead, age-adjusted death rates (AADR) per 100.000 person/years and incidence rate ratios (IRR) with 95% confidence intervals (95% CI) by sex and diabetes status, for cause of death.

Causes	Males					Females					Total				
	No diabetes		With diabetes			No diabetes		With diabetes			No diabetes		With diabetes		
	N	AADR (95% CI)	*N*	AADR (95% CI)	IRR (95% CI)	*N*	AADR (95% CI)	*N*	AADR (95% CI)	IRR (95% CI)	*N*	AADR (95% CI)	*N*	AADR (95% CI)	IRR (95% CI)
All causes (A00–T98)	4090	869.5 (843.3–895.6)	1240	1662.9 (1432.5–1893.2)	1.63 (1.52–1.73)	3090	600.5 (579.6–621.4)	788	1197.0 (1077.5–1316.4)	1.77 (1.64–1.92)	7180	730.9 (714.2–747.5)	2028	1422.7 (1295.3–1550.2)	1.68 (1.60–1.78)
CVD (I00–I99)	1161	249.8 (235.5–264.0)	357	432.5 (373.8–491.2)	1.56 (1.38–1.76)	1017	200.0 (187.8–212.2)	271	360.3 (315.0–405.5)	1.69 (1.47–1.93)	2178	224.1 (214.8–233.5)	628	395.3 (358.5–432.0)	1.61 (1.47–1.76)
AMI (I21–I23)	*221*	*46.4 (40.2–52.5)*	*57*	*70.9 (50.6–91.1)*	*1.48 (1.10–1.99)*	*137*	*26.9 (22.4–31.4)*	*39*	*51.6 (34.9–68.3)*	*1.81 (1.27–2.59)*	*358*	*36.3 (32.6–40.1)*	*96*	*60.9 (47.9–74.0)*	*1.59 (1.27–1.99)*
Other CVD	*950*	*203.4 (190.5–216.3)*	*300*	*361.6 (306.5–416.8)*	*1.58 (1.39–1.80)*	*880*	*173.1 (161.7–184.5)*	*232*	*308.6 (266.5–350.8)*	*1.67 (1.44–1.93)*	*1820*	*187.8 (179.2–196.4)*	*532*	*334.3 (299.9–368.8)*	*1.62 (1.47–1.78)*
Diabetes (E10–E14)	18	3.8 (2.0–5.6)	125	139.9 (114.2–165.6)	37.22 (22.61–61.30)	13	2.5 (1.1–3.9)	89	119.4 (90.9–147.9)	44.79 (24.98–80.31)	31	3.1 (2.0–4.2)	214	129.3 (110.1–148.6)	40.46 (27.68–59.14)
Renal causes (N00–N39)	87	19.0 (15.0–23.0)	25	26.1 (15.8–36.4)	1.37 (0.88–2.14)	59	11.5 (8.6–14.4)	21	26.0 (14.8–37.1)	2.37 (1.43–3.91)	146	15.1 (12.7–17.6)	46	26.0 (18.4–33.6)	1.71 (1.22–2.38)
Other causes	2824	596.9 (575.1–618.7)	733	1064.4 (842.9–1285.8)	1.44 (1.32–1.56)	2001	386.5 (369.7–403.4)	407	691.3 (584.5–798.1)	1.51 (1.35–1.68)	4825	488.5 (474.8–502.2)	1140	872.1 (751.5–992.7)	1.46 (1.37–1.56)

CVD = cardiovascular disease; AMI = acute myocardial infarction; AADR = age-adjusted death rate, using Italian population at 31.12.2009, stratified by sex.
IRR = calculated using Poisson model, adjusted for age, and sex. People without diabetes were used as reference.

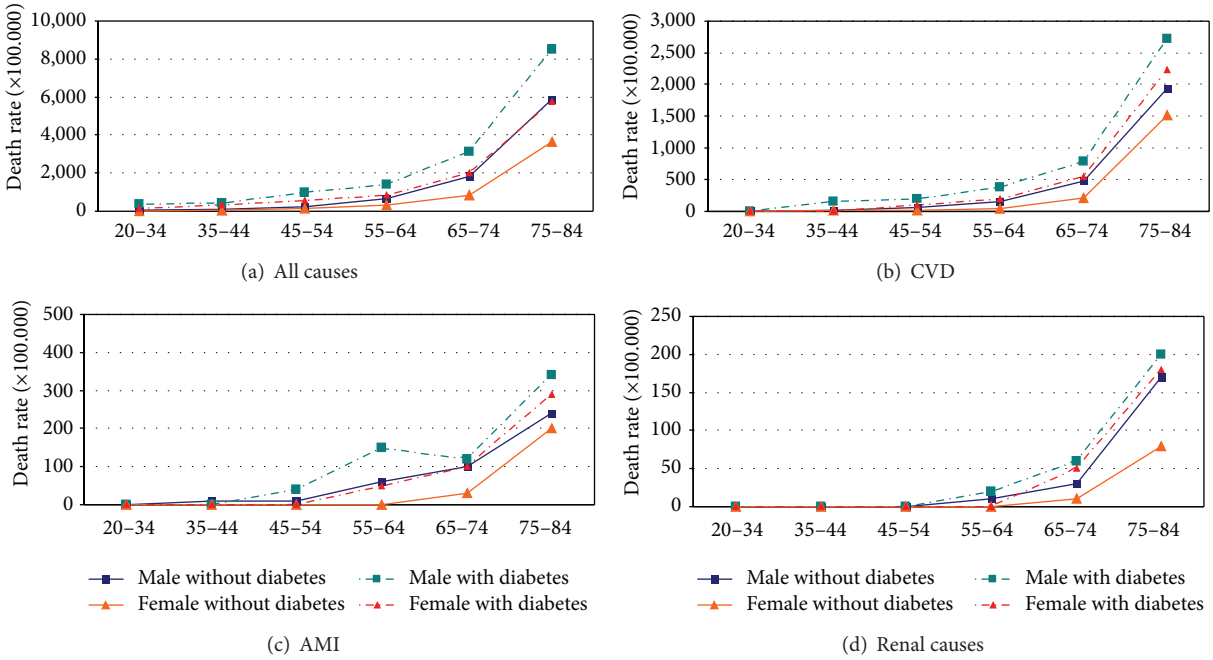

Figure 1: Age-specific death rates by sex and diabetes status: (a) all cause of death; (b) CVD; (c) AMI; (d) renal causes. The curves with dash indicate diabetic patients and the curve with squares indicate males and the curve with triangles indicate females.

and other disorders of the kidney and urethra. In this group, the deaths caused by renal failures were 70% of the total in the diabetic population, while the percentage decreased to 59% in nondiabetic population. In both subgroups, the remaining deaths were almost entirely ascribed to "other diseases of urinary system" block.

Comparing number of deaths by cause among diabetic and nondiabetic individuals, we observed 214 deaths caused by diabetes in the former subgroup and 31 in the latter (this subgroup included people with diabetes diagnosed after 2009), corresponding to a cause-specific age-adjusted death rate of 129.3 and 3.1 per 100000 p/y, respectively. The presence of the diabetes-specific cause makes it difficult to compare the other causes of mortality between the two populations. In fact, this cause of death subtracts cases to other causes and in particular to cardiovascular and renal causes, because often the final cause was attributable to one of these two categories.

Analysis of incidence rate ratios by age class suggests that the impact of diabetes decreases with increasing age (Table 4). The effect can only be observed in all-cause mortality and CVD as a whole, because the absolute AMI and renal causes risk of death are too small in younger ages. Nevertheless, the risk difference increased with age, reaching 26.3 per 1000 p/y in males aged 75–84 for all causes and 21.1 in females, while in 20–34-year-old class the difference was 1.6 per 1000 p/y in males and 1.3 in females. In case of CVD, the risk difference in the oldest age class reached 7 per 1000 p/y, in both sexes.

Comparison among age-specific death rates by sex and diabetes status (Figure 1) indicated that males with diabetes have the highest rates. However, females with diabetes have higher rates than males without diabetes mainly in the younger age groups, while females without diabetes have very low death rates until the age of 64.

4. Discussion

Our study found an excess of mortality associated with diabetes in both sexes, for all causes and for all groups of causes analyzed. However, the excess in the ratios was limited compared to findings of other studies [9, 27–29]. It must be emphasized that our study was population-based and data on exposure were retrieved from a register built using six different sources, assuring sensitivity and specificity [24]. This study design includes a wider denominator of exposed people compared to studies where the cohort is hospital or treatment based.

Focusing on CVD causes, the risk of death for diabetics is 61% higher than that for nondiabetics subjects (95% CI: 1.47–1.76), with no differences between the two subcategories, AMI and "other CVD causes".

Considering all causes of death, our study found evidence of greater impact of diabetes on females than males, despite the severity of disease seeming to be similar in the two groups, in agreement with a recent population-based retrospective cohort study [18]. When we analyzed CVD, IMA, and renal causes, the different effect of diabetes by sex was also present, although the power of the study does not permit ruling out the possibility that the difference was due to random fluctuations.

The reason why diabetes determines a greater excess of all-cause mortality in females than males is not completely understood, especially for CVD causes [30].

Table 4: Incidence rate ratios (IRR) with 95% confidence intervals (95% CI) and risk difference (RD) per 1000 p/y within age categories by sex, diabetics versus nondiabetic subjects, for all causes, CVD, AMI, and renal causes.

Age	All				CVD				AMI				Renal causes			
	Males		Females		Males		Females		Males		Females		Males		Females	
	IRR	RD	IRR	RD	IRR	RD	IRR	RD	IRR	RD	IRR	RD	IRR	RD	IRR	RD
20–34	8.03 (0.95–30.23)	3.1	7.02 (0.17–42.14)	1.4	—	—	—	—	—	—	—	—	—	—	—	—
35–44	3.68 (1.56–7.43)	2.9	5.60 (1.49–14.93)	2.7	6.60 (1.29–21.09)	1.3	—	—	—	—	—	—	—	—	—	—
45–54	3.86 (2.80–5.22)	7.1	3.83 (2.13–6.42)	4.0	4.14 (1.87–8.29)	1.4	6.73 (1.26–23.51)	0.8	2.65 (0.29–11.40)	0.3	—	—	—	—	—	—
55–64	2.18 (1.78–2.64)	7.5	2.51 (1.84–3.35)	5.0	2.51 (1.68–3.66)	2.3	4.50 (2.20–8.65)	1.6	2.29 (1.66–4.21)	0.9	9.88 (1.45–58.39)	0.5	2.67 (0.26–14.92)	0.1	—	—
65–74	1.72 (1.52–1.93)	13.1	2.32 (1.96–2.73)	11.3	1.62 (1.27–2.06)	3.0	2.68 (1.91–3.69)	3.4	1.13 (0.58–2.04)	0.2	3.26 (1.38–7.17)	0.7	1.88 (0.66–4.71)	0.3	4.48 (1.15–15.54)	0.4
75–84	1.45 (1.32–1.58)	26.3	1.58 (1.43–1.74)	21.1	1.40 (1.20–1.64)	7.8	1.47 (1.26–1.72)	7.2	1.45 (0.90–2.26)	1	1.45 (0.91–2.25)	0.9	1.17 (0.63–2.05)	0.3	2.25 (4.10–6.07)	1

One explanation is that type-2 diabetes mellitus (T2DM) may reduce the advantage of females in the prevalence of cardiovascular disease by fading the vascular protective effects given by estrogens [21, 31–33]. Many authors have suggested that the CVD risk factors have a stronger impact on females than males [14, 15, 32–34]. Compared to males, females with diabetes have a worse cardiovascular profile, which could explain their higher cardiovascular mortality, mainly at age <60. Females with diabetes have higher prevalent abdominal obesity [34, 35], increasing the risk of hypertension [19, 36], a worse lipid profile, since the onset of diabetes (low levels of HDL cholesterol [HDL-C], small particle size of LDL cholesterol [LDL-C], and high levels of triglycerides) [35, 37–39], and a more marked endothelial dysfunction than males with diabetes [40–45], a greater degree of fibrinolysis/thrombosis compared to males [46, 47], and also an increased prevalence of hypoglycemic events compared to that of male diabetic patients [48]. These phenomena might explain the increased incidence of cardiovascular events and mortality among female patients [49].

Besides innate differences in sex physiology, disparities between sexes in the treatment of major cardiovascular risk factors also exist [35, 50, 51]. These can be attributed to an underestimation of patient risk and a less aggressive approach (i.e., prescription of lower doses) and poorer compliance of females [52–55]. Nevertheless, two Italian studies did not find any relevant differences between females and males in terms of the quality of diabetes care [35, 56]. In one of these recent large cross-sectional studies, women were less likely to reach the recommended targets despite receiving the same treatment for lipid control and hypertension and they were more likely to be overtreated with insulin. Women still showed a lower likelihood of being monitored for diabetes complications, particularly foot and eye complications. As for intermediate outcomes, the proportion of individuals reaching the targets of HbA1c, LDL cholesterol, and BMI values was systematically lower for women than men. The only result that went in the opposite direction was that, among diabetic patients with high LDL cholesterol, a higher proportion of women were not treated with lipid-lowering therapy [35].

In our study we also investigated mortality caused by renal diseases and in particular codes N00-N39, that is, glomerular diseases, renal tubulointerstitial diseases, acute kidney failure and chronic kidney disease, urolithiasis, other disorders of kidney and urethra, and other diseases of the urinary system, given the close interconnection between renal and cardiovascular disease.

For this group as well, we found risk excess in diabetic population and the excess was stronger in females than males. This excess in females is closely linked to CVD mortality and could partially explain its increase [57–59]. There are few sets of data on the role of gender on microvascular complications and increasing mortality related to them [60]. While females in the general population have less renal disease, this advantage is less evident in diabetic nephropathy than nondiabetic kidney diseases [61–63]. The contribution of sex to diabetic renal disease is still unclear. Although some studies indicate that females progress at a faster rate [64], others studies indicate the opposite [65–67]. Some studies suggest that male sex remains a risk factor for the development of micro- and macroalbuminuria as well as the progression of an established diabetic nephropathy [68]. However, the prevalence of a reduced glomerular filtration rate estimated in females was higher than that in males [69]. This nonalbuminuric renal impairment phenotype is associated with higher incidence of CVD, particularly in the coronary district [70].

The differences in therapy effectiveness in females as well as the existence of different disease pathways in the kidney and cardiovascular disease have led some authors to suggest the need to develop gender-specific therapeutic strategies to prevent renal dysfunction and reduce associated morbidity and mortality in females [71].

Nevertheless, it is important to note that even if the incidence rate ratios declined with age, the risk difference increased in the older groups, where the number of deaths is much higher. In other words, in a hypothetical population free of diabetes disease, in age class 75–84 years, 26 deaths for every 1,000 males and 21 deaths for 1,000 females still alive would be avoided, while the savings would be 1.6 for 1,000 males and 1.3 for 1,000 females in the age class 20–34 years.

The particularly high excess of risk in younger ages is mainly due to low mortality in nondiabetic group, and the phenomenon is more pronounced in females. Our results agree with other studies [69, 70].

5. Strengths and Limitations

This is a population-based cohort study using data from a province-wide diabetes register for exposure identification and from mortality register for case detection, thereby reducing misclassification bias. Moreover, while there have been several studies on all causes and CVD mortality among people with diabetes, this is one of the few studies exploring the effect of diabetes on renal causes mortality. Finally, our study focused on the greater impact of diabetes on female mortality, exploring possible hypotheses for this phenomenon.

However, this study considered only age as confounder; other possible confounders, such as socioeconomic characteristics, behavioral risk factors (i.e., BMI, smoking), and clinical information other than treatment, such as duration of disease and micro- and macrovascular diabetes complications, were not considered.

Finally, the presence of diabetes as cause of death makes it difficult to compare the cause-specific mortality between the population with and without diabetes, in particular for CVD and renal diseases.

6. Conclusions

Diabetes determines a 68% excess in mortality rate. The relative risk for diabetic patients versus nondiabetic population is particularly relevant in young and middle-aged subjects, where diabetes status contributes to occurrence of deaths that are unexpected in nondiabetic population. Furthermore, diabetes has a greater impact on females than males, reducing

the advantage of females in all-cause mortality as well as CVD, in particular AMI, and renal mortality observed in the population without diabetes.

Further studies are needed to determine whether the difference in cardiovascular risk profile or the quality of care delivered justifies the higher excess of mortality in females with diabetes than males.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Hidden Metabolic Disturbances in Women with Normal Glucose Tolerance Five Years after Gestational Diabetes

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Background. The study aimed to assess whether women with prior gestational diabetes (pGDM), despite maintenance of normal glucose tolerance (NGT) five years after delivery, display metabolic disturbances compared to healthy controls. *Methods*. 45 pGDM with NGT were compared to 18 women without a history of GDM (CON), matched for age (37.0 ± 4.1 versus 35.2 ± 5.3, P = ns) and BMI (24.3 ± 3.1 versus 23.3 ± 3.3, P = ns). Metabolic parameters were derived from oral and intravenous glucose tolerance tests; furthermore lipid profile, C-reactive protein (CRP), adiponectin, leptin, and glucagon were assessed. *Results*. Five years postpartum, pGDM had increased glucose concentrations during the OGTT (AUC: 1.12 ± 0.15 versus 1.0 ± 0.12 mol/L $*$ min, $P = 0.003$) and insulin sensitivity was decreased compared to CON (OGIS: 467.2 ± 64.1 versus 510.6 ± 53.1 mL/min $*$ m^2, $P = 0.01$). pGDM had lower adiponectin (8.1 ± 2.6 versus 12.6 ± 5.3, $P < 0.008$) but increased waist circumference and CRP compared to CON. *Conclusions*. Despite diagnosis of normal glucose tolerance, pGDM are characterized by hyperglycemia and insulin resistance compared to healthy controls, accompanied by decreased adiponectin and increased CRP concentrations, thus linking metabolic disturbances to an increased cardiovascular risk in pGDM.

1. Background

Gestational diabetes mellitus (GDM) identifies women with an increased risk of developing type 2 diabetes and cardiovascular disease. Therefore, women with prior GDM (pGDM) are recommended to regularly undergo assessment of glucose tolerance [1] in order to detect overt diabetes in time to initiate treatment to prevent complications.

The current view of GDM pathophysiology is that women who develop GDM are characterized by "*beta-cell dysfunction based on chronic insulin resistance*" [2]. Consequently, disturbances in glucose metabolism are thought to be of chronic nature rather than of acute onset during pregnancy [3].

We have previously shown that pGDM with metabolic deterioration within five years after delivery are characterized by hyperglycemia, insulin resistance, and hyperinsulinemia; furthermore first-phase insulin secretion was impaired in these women compared to pGDM who did not experience deterioration of glucose tolerance [4]. In addition, while those pGDM who progress to diabetes have a marked increase in glycemia and insulin resistance before diabetes onset, we observed that beta-cell function declines continuously over years [5]. Others have shown that declining beta-cell compensation for increased insulin resistance (estimated by the disposition index) characterizes pGDM who convert to overt diabetes within 12 years after delivery [6] and that this decline was associated with an increase in body weight and C-reactive protein (CRP) as well as a decrease in adiponectin [7].

Approximately 50% of pGDM develop type 2 diabetes within the first five years after delivery [8, 9]. At the same time, there is a group of pGDM who are able to maintain

normal glucose tolerance within this vulnerable period of metabolic deterioration. Although many studies describe metabolic changes in those who convert to diabetes, less is known about pGDM who are able to maintain normal glucose tolerance (NGT) over the years following delivery. It could be speculated that metabolic disturbances are cured in those pGDM and that their risk of developing diabetes equals the one of women without a history of GDM. Consequently, time- and cost-consuming follow-ups would not be necessary in these women. This could help to reduce unnecessary costs.

Since data that would answer this question are scare, the aim of this study was to compare validated parameters of insulin sensitivity and secretion between pGDM with NGT five years after gestational diabetes and healthy controls (five years after normal pregnancy). In addition, metabolic changes in pGDM within the five-year observational period and the impact of weight loss within this time were investigated.

2. Study Population and Methods

2.1. Study Population. The current study was part of the Viennese Post-Gestational Diabetes Project (VPGDP), a prospective longitudinal study in women with a history of GDM. pGDM were recruited during a pregnancy complicated by GDM in the Diabetes Outpatient Clinic of the Division of Endocrinology and Metabolsim of the Medical University of Vienna, where they had been seen during pregnancy. Healthy controls (CON) were recruited from the department of obstetrics and gynecology. The Human Ethics Committee of the Medical University of Vienna approved the protocol and all women gave written informed consent.

Exclusion criteria were age ≤ 18 years, known preexisting glucose intolerance (impaired glucose tolerance, type 1 or type 2 diabetes), diagnosis of GDM before the 8th gestational week, positive diabetes-associated antibodies (assessed during and after pregnancy at our division), ethnicity other than Caucasian, morbid obesity (pregestational body mass index > 40 kg/m^2), or evidence of chronic diseases, including kidney or liver diseases and chronic inflammatory diseases. In CON, exclusion criteria additionally included the existence of any risk factors for diabetes (i.e., positive family history or chronic medication known to influence carbohydrate metabolism).

For the current study, 45 pGDM with normal glucose tolerance at five-year follow-up and 18 healthy controls were matched for age and body mass index (Table 1). Data from the visit 5 years postpartum were compared between the groups and in pGDM also to the baseline examination (six months after delivery). Furthermore, an additional subanalysis was performed, in which those pGDM with weight loss of ≥7% (wihtin the 5-year observational period) were compared to the healthy control group. This range was chosen in regard to the American Diabetes Association (ADA) recommendation of 7% weight loss in patients with prediabetes [10].

2.2. Methods. All women received dietary counseling and were recommended to regain normal body weight by intake of a healthy diet and regular physical activity. Glucose tolerance tests were scheduled between day 3 and day 10 of their menstrual cycle, performed in the morning after an overnight fast of at least 8 hours. Women were asked to refrain from physical activity 3 days prior to the follow-up visits. All women underwent an oral glucose tolerance test (OGTT) and the majority underwent also an intravenous glucose tolerance test (76% of pGDM and 61% of CON). Reasons for not undergoing an intravenous glucose tolerance test (IVGTT) were mainly problems in time scheduling (additional appointment according to menstrual cycle, more than 2 weeks after OGTT).

Table 1: Baseline characteristics in women with prior gestational diabetes (pGDM) and healthy controls (CON).

	pGDM	CON	*P* value
Age (years)	37.0 ± 4.1	35.2 ± 5.3	ns
Body mass index (kg/m^2)	24.3 ± 3.1	23.3 ± 3.3	ns
Waist circumference (cm)	81.8 ± 9.8	73.6 ± 8.3	<0.005
HbA1C (%)	5.3 ± 0.3	5.3 ± 0.2	ns
Fasting plasma glucose (mg/dL)	88.2 ± 5.6	84.0 ± 6.2	0.01

2.2.1. Oral Glucose Tolerance Test (OGTT). After a venous catheter was placed into an antecubital vein, blood samples for the measurement of glucose, insulin, and C-peptide were taken at fasting as well as 10, 20, 30, 60, 90, 120, 150, and 180 minutes after ingestion of 75 g glucose in a solution.

2.2.2. Intravenous Glucose Tolerance Test (IVGTT). For the IVGTT, one venous catheter for blood sampling was placed in one antecubital vein and another one for intravenous administration of glucose and insulin in an antecubital vein of the other arm. Blood samples (for measurement of glucose, insulin, and C-peptide) were drawn at the fasting state (−10 and 0 minutes) and 3, 4, 5, 6, 8, 10, 14, 19, 22, 27, 30, 35, 40, 50, 70, 100, 140, and 180 minutes after injection of glucose (300 mg/kg body weight). At 20 minutes, normal insulin (Humulin R, Eli Lilly, Indianapolis, IN, USA) was given with a concentration of 0.03 IU/kg body weight and for a duration of 5 minutes [11].

2.2.3. Assessment of Metabolic Parameters, Lipids, and Cardiovascular Biomarkers. Glucose was immediately analyzed by the hexokinase method in our central lab. Serum samples for the assessment of insulin and C-peptide were immediately cooled down, centrifuged, stored at −20 degrees Celsius, and later analyzed in the lab of the Division of Endocrinology and Metabolism by commercially available radioimmunoassay kits: insulin (Serono Diagnostics, Freiburg, Germany) and C-peptide (CIS Bio International, Cedex, France) with interassay coefficients of variation of <5%.

At fasting, additional blood samples were taken and the following parameters were assessed: adiponectin was measured in duplicate by an ELISA system developed for

the assessment of human plasma adiponectin concentrations (Department of Internal Medicine and Molecular Science, Osaka University, Suita, Osaka, Japan) [12]. Leptin (Human Leptin RIA kit; Linco Research, St. Charles, MO, USA) and glucagon (ICN Biomedicals, Costa Mesa, CA, USA) were measured in duplicate by commercially available radioimmunoassay kits with a CV < 6% for leptin and <8% for glucagon.

HbA1C (by high-performance liquid chromatography, given in % [13]), TSH, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and C-reactive protein [14] were measured by established methods in the Central Lab of the Medical University of Vienna.

2.2.4. Data Analyses. Normal glucose tolerance (NGT) was defined according to the criteria of the ADA [10]: fasting plasma glucose (FPG) < 100 mg/dL and 2-hour post-OGTT glucose < 140 mg/dL.

The oral glucose insulin sensitivity (OGIS) index describes glucose clearance per unit change of insulin concentration [15]. Parameters of insulin secretion were described by the areas under the curve (AUC) of insulin and C-peptide during OGTT and IVGTT, calculated with the trapezoidal rule. Hepatic insulin extraction (HIE, given in %) was quantified with a mathematical model of insulin/C-peptide interactions [16].

From IVGTT, insulin sensitivity index (S_I, in 10^{-4} min^{-1}/(μU/mL)) describing insulin effect on glucose disappearance [17] was computed. First-phase insulin secretion was assessed by $\Delta\text{AIR}_\text{G}$ calculated by averaging insulin concentrations above basal from 3 to 10 minutes and given in μU/mL. The disposition index derived from IVGTT (10^{-2} min^{-1} [18]) was calculated as $S_I \times \Delta\text{AIR}_\text{G}$ and describes the combined effect of insulin secretion and sensitivity on glucose disposal [19]; it is frequently used to describe the ability of the beta cells to adapt for increased insulin resistance.

2.2.5. Statistical Analyses. Between group differences were calculated by ANOVA; changes between baseline and follow-up visit in pGDM were calculated by a paired t-test. Associations between continuous variables are described by Pearson's correlation coefficient. Data are given in means ± standard deviation. Levels of significance were set at $P < 0.05$. SAS software (Enterprise Guide 4.3, SAS Institute Inc., Cary, NC, USA) was used for all computations.

3. Results

3.1. Metabolic Differences between pGDM and CON Five Years Postpartum. Five years after the index pregnancy, pGDM—despite normal glucose tolerance—had significantly higher levels of plasma glucose at fasting as well as 60 minutes of the OGTT compared to CON; in line, the AUC of glucose was significantly increased in pGDM compared to CON (Figure 1). Insulin sensitivity, derived by OGIS, was decreased in pGDM compared to CON (Figure 2(a)). Furthermore, adiponectin was lower in pGDM (Figure 2(b)), while blood pressure, leptin, glucagon, TSH, and lipid profile did not differ between the groups. pGDM had significantly higher waist circumference (Figure 2(c)) as well as CRP concentrations, (Figure 2(d)). Normal body weight, defined as BMI < 25 kg/m^2, was found in 30 out of 45 pGDM (=66.7%) and in 14 out of 18 CON (=77.8%). Insulin sensitivity (OGIS) was negatively correlated with BMI and body weight in the whole study group (BMI: $R = -0.3$, $P = 0.01$; body weight: $R = -0.3$, $P = 0.02$).

3.2. Metabolic Changes in pGDM within the Five-Year Follow-Up Period. When follow-up data in pGDM were compared to their baseline examination (= six months postpartum), waist circumference (85.5 ± 9.0 versus 81.8 ± 9.8 cm, $P = 0.002$) and diastolic blood pressure (76.7 ± 9.1 versus 72.5 ± 9.4 mmHg, $P < 0.04$) were lower at five-year follow-up compared to baseline (6 months after pregnancy). In addition, pGDM had significantly lower total cholesterol (from 211.9 ± 48.6 to 198.4 ± 40.2 mg/dL, $P < 0.006$) and LDL-cholesterol (from 134.2 ± 45.4 to 122.6 ± 37.0 mg/dL, $P = 0.002$) compared to baseline. No change was observed in body weight, HbA1C, glucagon, leptin, or TSH.

Furthermore, the disposition index (1.7 ± 1.2 versus 2.6 ± 2.3 10^{-2} min^{-1}, $P < 0.004$) and insulin sensitivity derived from IVGTT (from 4.3 ± 2.5 to 6.3 ± 3.2 10^{-4} min^{-1}/(μU/mL), $P = 0.001$) were increased compared to baseline.

3.3. The Impact of Weight Loss on Metabolic Status at 5 Years Postpartum. Twelve pGDM had significant weight loss (≥7%) within the 5-year observational period (pGDM_wl) and were compared to CON, in order to assess whether weight loss was associated with an improved metabolic profile. While there was no difference in age, BMI, and waist circumference between the groups, pGDM_wl had significantly increased concentrations of glucose (AUC of glucose, 1.13 ± 0.16 versus 1.0 ± 0.12 mol/L ∗ min, $P < 0.02$) and insulin during the OGTT (TIS: 27.1 ± 7.1 versus 21.8 ± 5.7 nmol/L, $P = 0.03$, AUC of C-peptide: 419.5 ± 106.9 versus 347.0 ± 81.3 nmol/L ∗ min, $P = 0.04$), whereas insulin sensitivity was lower compared to CON (OGIS: 466.9 ± 46.4 versus 510.6 ± 53.1 mL/min ∗ m^2, $P < 0.03$). Furthermore, CRP was higher in pGDM_wl compared to CON (0.4 ± 0.3 versus 0.2 ± 0.2 mg/dL, $P = 0.04$).

When pGDM_wl were compared to their baseline state, the significant changes in body weight (−9.9 ± 4.8 kg, BMI: from 26.8 ± 3.5 to 23.2 ± 2.5 kg/m^2, both $P < 0.0001$) and waist- (from 89.0 ± 7.9 to 79.8 ± 9.4 cm, $P = 0.0002$) and hip-circumference (from 107.5 ± 5.8 to 97.8 ± 4.6 cm, $P < 0.0001$) were accompanied by an improved disposition index (from 1.6 ± 1.0 to 2.9 ± 1.6 10^{-2} min^{-1}, $P = 0.01$; in line with the whole pGDM-group), a decline in CRP concentrations (from 0.6 ± 0.2 to 0.4 ± 0.3 mg/dL, $P = 0.03$), diastolic blood pressure (from 80.8 ± 9.3 to 72.2 ± 7.9 mmHg, $P = 0.02$), and leptin (from 17.7 ± 5.5 to 14.5 ± 7.0 ng/mL, $P < 0.03$).

4. Discussion

The current study aimed to assess whether disturbances in glucose metabolism can be observed in women with prior

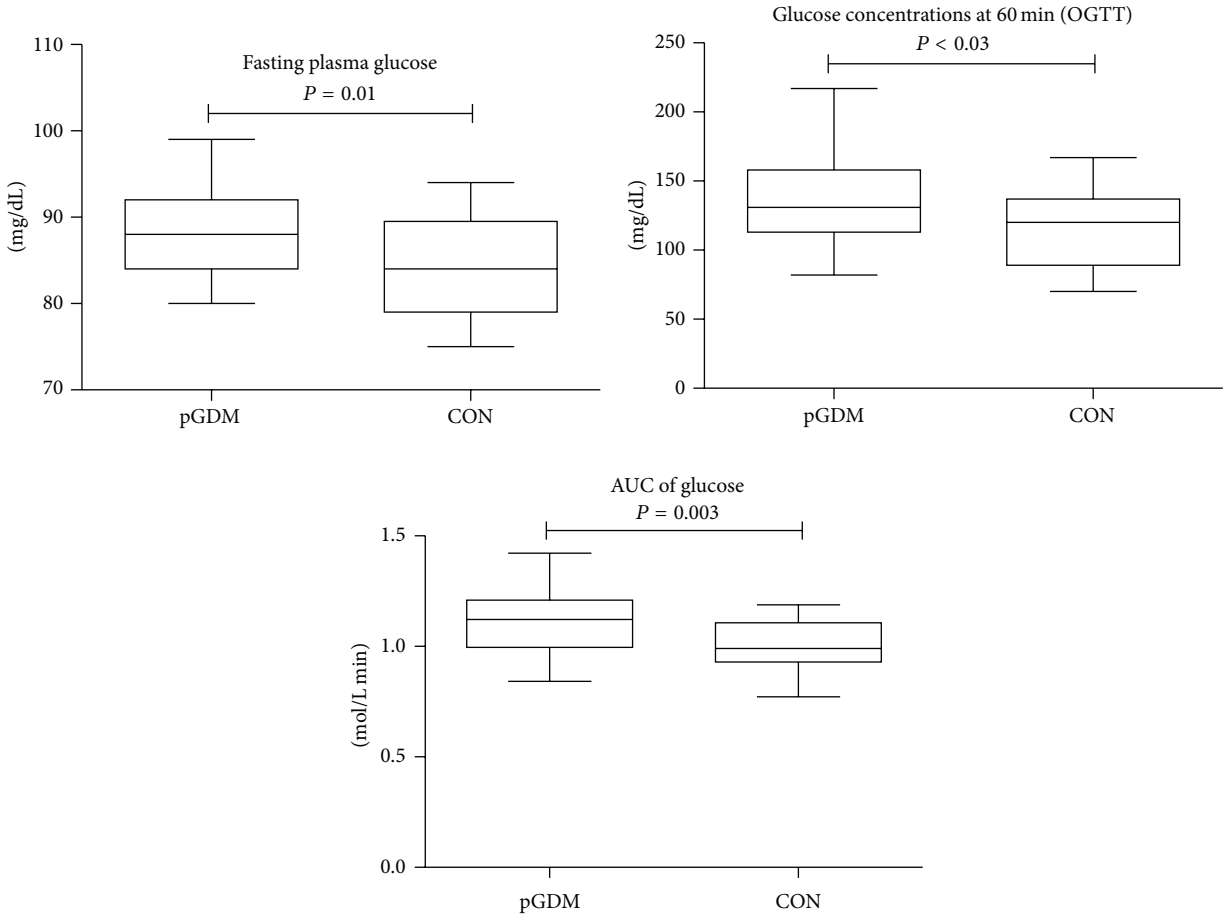

Figure 1: Higher glucose concentrations at fasting and stimulated conditions in pGDM compared to CON.

gestational diabetes (pGDM) who were able to maintain normal glucose tolerance (NGT) until five years after a GDM-pregnancy. According to our data, pGDM—despite normal glucose tolerance—were still characterized by decreased insulin sensitivity and increased glucose concentrations during the OGTT compared to healthy controls. Furthermore, CRP levels and waist circumference were higher in pGDM compared to CON, despite comparable BMI, while adiponectin was decreased in pGDM. In addition, pGDM with weight loss ≥ 7% within the five-year follow-up period exhibited pronounced metabolic disturbances compared to CON.

Insulin resistance is a frequent finding in women with prior gestational diabetes and associated with ectopic lipid accumulation in skeletal muscle and liver [20, 21]. It is assumed that gestational diabetes develops on the background of chronic insulin resistance, aggravated by the physiological insulin resistance of late pregnancy [2, 3]. According to our observations, we also assume that insulin resistance in pGDM is of chronic nature and the diagnosis of GDM during pregnancy detects a metabolic phenotype with increased insulin resistance in a young female cohort.

We found a weak, but significant, inverse association between BMI and insulin sensitivity; however, also in the subgroup of pGDM with significant weight loss (pGDM_wl) insulin sensitivity was significantly lower compared to the healthy control group. Special attention has to be given to this subgroup of pGDM who had significant weight loss of ≥7% within the follow-up period. This group had a BMI of approximately 27 kg/m^2 six months after delivery and five years postpartum mean BMI was 23.2 ± 2.5 kg/m^2. Weight loss in this group was accompanied by a reduction in waist- and hip-circumferences as well as leptin; furthermore, the disposition index (in line with the whole study group) improved; however, despite marked changes in body weight, insulin sensitivity and glucose concentrations during the OGTT and IVGTT remained unchanged compared to the baseline examination (6 months postpartum). Hence, it might be speculated that obesity is not the main reason for insulin resistance in pGDM. This assumption is supported by one of our prior observations, which showed that insulin resistance is pronounced in lean subjects with GDM and persists after delivery [22]. In addition, it is in line with prior investigations in women with a history of GDM showing that the decline in insulin sensitivity and beta-cell compensation could not be explained by changes in adiposity [23]. It has also been shown that impaired beta-cell glucose sensitivity independent of obesity and hyperglycemia displays a risk factor in pGDM [24].

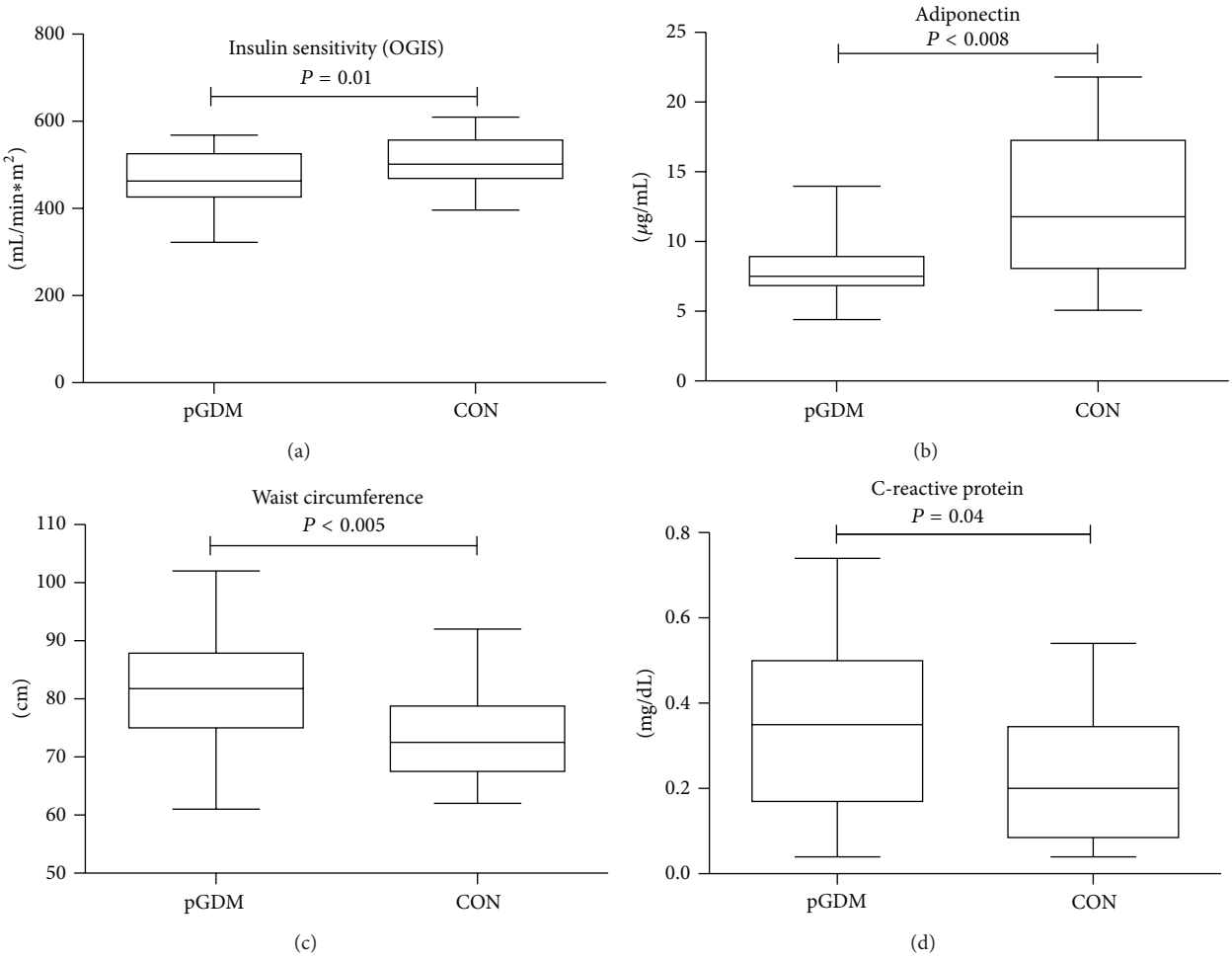

Figure 2: Insulin sensitivity (OGIS) and adiponectin were decreased in pGDM, while waist circumference and CRP were increased compared to CON.

It has to be noted that the majority of women in our study groups (66.7% in pGDM and 77.8% in CON) had a BMI lower than 25 kg/m^2 and thus fulfill the criteria of normal body weight. Furthermore, mean waist circumference in pGDM was 81.8 cm, which would fulfill the WHO criteria for metabolic syndrome, but not those of the US National Cholesterol Education Program Adult Treatment Panel III [25]. This again leads us to conclude that obesity might not be the main trigger of glucose intolerance in this young female cohort or not in all pGDM.

The observation that glucose concentrations during the OGTT were higher in pGDM compared to CON clearly indicates a risk of hyperglycemia in these women. This risk would not have been detected by simply concentrating on the definition of NGT, because glucose values were within the normal range; however, in comparison to age- and BMI-matched controls, glucose concentrations during the OGTT were significantly higher in pGDM. And even in pGDM_wl, the significant weight loss did not counteract increased glucose concentrations compared to CON.

The finding that adiponectin was decreased and CRP increased in pGDM compared to CON is of great interest. A recent study by Xiang and coworkers [6, 7] described that declining beta-cell compensation in pGDM (described by the disposition index) is—besides weight gain—associated with declining levels of adiponectin and rising CRP levels. To our surprise, these metabolic features were also observed in our group of pGDM who were able to maintain normal glucose tolerance within this vulnerable period of five years postpartum. Hence, the question of whether these metabolic alterations can be used as markers of metabolic deterioration or simply reflect this special metabolic profile in pGDM appears. Specifically a drop in adiponectin could reflect metabolic deterioration in pGDM and indicate the need for closer follow-up of these women; however, this assumption has to be reexamined in future prospective studies.

Chronic inflammation and hypoadiponectinemia are frequently found in patients with diabetes; hence it appears that these disturbances could be the cause rather than the consequences of insulin resistance and hyperglycemia. In addition, this combination of hypoadiponectinemia and increased CRP might contribute to the development of atherosclerosis [26, 27]. Hence, we could assume that pGDM despite normal glucose tolerance have an increased cardiovascular risk and

should be considered as high-risk population for cardiovascular disease.

Several metabolic parameters, that is, LDL-cholesterol, diastolic blood pressure, and waist circumference as well as the disposition index and insulin sensitivity derived from IVGTT, were improved in pGDM at five years postpartum compared to baseline. But despite these ameliorations and the fact that the majority of our pGDM group were able to regain/maintain normal body weight and normal glucose tolerance, disturbances in glucose regulation were observed. Hence, it appears that the metabolic profile and thus the risk of developing type 2 diabetes seem to be chronic and that GDM only identifies women at risk. It appears that the pivotal mechanisms that finally lead to the development of overt hyperglycemia in pGDM have not been elucidated in detail. This may include a genetic disposition in this group of GDM without obesity but still increased risk for type 2 diabetes. As shown, GDM risk is associated with genes involved in the regulation of insulin secretion [28]. At least, more studies are needed to better understand the development of overt hyperglycemia and develop treatment strategies, which can improve prevention.

While the strength of the current study lies in the investigation of a well-characterized cohort and performance of validated tests under dynamic—not only fasting—conditions, it also has some limitations: the study group is quite small and follow-up is limited to five years; an extended follow-up period could allow strengthening our conclusions.

Consequently we can summarize that metabolic disturbances which predispose pGDM to the development of overt diabetes appear to be chronic and can be hidden but, still, remain life-long and therefore regular follow-ups should be recommended to all women with a history of GDM in order to detect diabetes in time and prevent complications, especially the onset of cardiovascualr disease.

Conflict of Interests

The authors declare no conflict of interests related to this study.

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Research Article

Urinary Angiopoietin-2 Is Associated with Albuminuria in Patients with Type 2 Diabetes Mellitus

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Aims. To evaluate the levels of angiopoietin-1 (Ang-1), Ang-2, and vascular endothelial growth factor (VEGF) in serum and urine, and their association with albuminuria in patients with type 2 diabetes mellitus. *Methods*. In 113 type 2 diabetic patients with normoalbuminuria, microalbuminuria, and macroalbuminuria and 30 healthy controls, the levels of Ang-1, Ang-2, and VEGF in serum and urine were measured by enzyme-linked immunosorbent assay (ELISA). *Results*. Urinary and serum levels of Ang-2 were significantly higher in diabetic patients with normoalbuminuria than in healthy controls. Increased urinary Ang-2 level was positively associated with the degree of albuminuria. Urinary Ang-1 levels were significantly higher in normoalbuminuria patients and lower in macroalbuminuria patients than in controls. The levels of urinary VEGF increased in the albuminuria subgroup, though serum levels of Ang-1 and VEGF did not change. Urinary Ang-2 levels were correlated positively with albuminuria and negatively with glomerular filtration rate (GFR). Stepwise multiple regression analysis identified albuminuria ($P < 0.001$) and GFR ($P = 0.001$) as significant predictors of urinary Ang-2. *Conclusions*. Our data suggest that urinary Ang-2 is stepwise increased with renal damage in patients with type 2 diabetes mellitus and is associated with albuminuria.

1. Introduction

Diabetic nephropathy (DN) is a common complication of type 2 diabetes mellitus (DM) and is the leading cause of end-stage renal disease (ESRD). Microalbuminuria is an early sign of DN, which correlates with and can predict the progression of renal damage and cardiovascular morbidity [1–5]. The discovery of biomarkers for the earlier stages of DN would enable early intervention to reduce the impact of this chronic vascular complication.

Diabetic nephropathy is associated with altered vascular structure, endothelial dysfunction, and disrupted homeostasis and angiogenesis [4, 6, 7]. Abnormal glomerular angiogenesis in patients with DN is associated with glomerular hypertrophy and results in glomerular capillary injury and urinary albumin excretion [8, 9]. Thus, the mechanisms for the development of abnormal angiogenesis in DN involve a complicated interplay between pro- and antiangiogenic factors. Two families of growth factors, angiopoietin/Tie-2 and vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR), are thought to be associated with the development of DN. VEGF increases vascular permeability and is mitogenic for endothelial cells, acting early and at most points of the angiogenic cascade [10]. Within the angiopoietin family, angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) are the best-studied ligands for Tie-2 receptors. Ang-1 signaling via Tie-2 is involved in capillary sprouting, endothelial cell survival, and vascular remodeling [11]. Ang-2 is a natural antagonist of Ang-1 [12, 13]. Several studies suggest that Ang-2 signaling, in combination with VEGF, leads to sprouting angiogenesis, while Ang-2 signaling in the absence of VEGF causes vessels to regress [11]. Hence, selective upregulation of

VEGF and Ang-2 may lead to aberrant proliferation of leaky, friable vessels [10]. Emerging evidence suggests that VEGF and angiopoietin are critical in glomerular physiology and in the pathogenesis of glomerular disease in DM [14–17].

Previous studies have reported upregulated plasma levels of VEGF and Ang-2 in human and animal DN [10, 16, 18–20]. Notably, circulating Ang-2 levels associated with albuminuria have been reported in chronic kidney disease (CKD) [5] and systemic lupus erythematosus (SLE) [21]. Transgenic mice with inducible overexpression of Ang-2 in podocytes have been shown to develop significant increases in albuminuria [22], which, in turn, correlates with and predicts the progression of renal damage in DM [23]. Increased Ang-2 levels in patients with DM are associated with indexes of endothelial damage and dysfunction [16]. However, little is known about the urinary levels of these angiogenic factors in different stages of DN, and potential correlations between them and albuminuria have not yet been studied. In the present study, we measured urinary Ang-2, Ang-1, and VEGF levels to elucidate the possible correlation between urinary angiogenic factors and renal damage in patients with various phases of type 2 DM.

2. Materials and Methods

2.1. Subjects. The retrospective study included 113 insulin-dependent patients with type 2 DM recruited from the Department of Endocrinology and Nephrology at Union Hospital (Wuhan, China) between December 2012 and March 2014. Type 2 DM was defined according to established WHO criteria [24] (i.e., fasting blood glucose (FBG) ≥ 7.0 mmol/L, postprandial blood glucose ≥ 11.1 mmol/L, or symptoms of DM with random blood glucose ≥ 11.1 mmol/L). The mean duration of DM was 10.33 years (1 month–30 years). All patients maintained a stable body weight for at least 3 months before beginning the study. None of the patients had evidence of acute diabetic complications. Patients with acute vascular events or hospitalization (defined as stroke, myocardial infarction, unstable angina, or coronary or peripheral revascularization within the last 3 months) [16]; high-range hypertension (≥160/100 mmHg); current infection; or evidence of neoplastic, hepatic, or significant renal disease (requiring dialysis) within 3 months prior to enrollment were excluded from the study [16]. Patients requiring treatment with glucocorticoids or other drugs affecting glucose metabolism were also excluded. Diagnosis of DN was made according to the criteria of Kidney Disease Outcomes Quality Initiative (KDOQI) [25]. Based on the urinary albumin excretion rate (UAER) at baseline, patients were classified as having normoalbuminuria (DN1 group: UAER < 20 μg/min), microalbuminuria (DN2 group: UAER 20–200 μg/min), or macroalbuminuria (DN3 group: UAER > 200 μg/min). Thirty subjects undergoing routine health checks were recruited into the control group (NC). The study protocol was approved by the Medical Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, and was conducted according to the principles of the Declaration of Helsinki. Patients and control subjects provided written informed consent to participate in the study.

2.2. Laboratory Measurements. Morning preprandial levels of fasting blood sugar (FBG), serum creatinine, triglyceride (TG), high-density lipoprotein cholesterol (HDL-ch), low-density lipoprotein cholesterol (LDL-ch), and total cholesterol were measured with a full automatic biochemical analyzer (Hitachi 7150, Tokyo, Japan). Glycosylated hemoglobin (HbA1c) was measured with a D10 hemoglobin testing system (Bio-Rad Laboratories, Hercules, CA, USA), using a cation exchange HPLC and an immunoturbidimetric assay method (Roche/Hitachi 902 Cobas System). Twenty-four h urine samples were collected for the determination of Ang-1, Ang-2, and VEGF levels and the estimation of UAER. Urinary protein quantitative measurements (24 h UPQM) were obtained for all patients. Renal function was assessed by glomerular filtration rate (GFR) detection with single photon emission computed tomography (SPECT) (GE Millennium MG Dualhead, GE Healthcare, Milwaukee, WI, USA, USA). Vital signs and body mass index (BMI) were recorded.

2.3. Quantification of Ang-1, Ang-2, and VEGF. Levels of angiogenic factors (Ang-1, Ang-2, and VEGF) in serum and urine were measured by enzyme-linked immunosorbant assay (ELISA) in frozen serum samples and in urine samples. In brief, monoclonal antibodies specific for Ang-1 (Raybiotech, Norcross, GA, USA), Ang-2 (Raybiotech), and VEGF (NeoBioscience Technology Co., Shenzhen, China) were precoated onto microplates. Standards and samples were pipetted into the wells. Ang-1, Ang-2, or VEGF present in a sample was bound to the wells by the immobilized antibody. The wells were washed, and biotinylated anti-human antibody specific for Ang-1, Ang-2, or VEGF was added. After the removal of unbound antibodies, HRP-conjugated streptavidin was pipetted into the wells. The wells were washed, and a 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution was added. The TMB solution changed in color from blue to yellow, in proportion to the bound concentration of Ang-1, Ang-2, or VEGF. The absorbance of the solution in each well was measured by a microplate reader (Bio-Tek ELx800; VT, USA) at a wavelength of 450 nm. All samples were examined in duplicate, and mean values were used for statistical analysis.

2.4. Statistical Analysis. Statistical analysis was performed using a commercially available statistical software package (SPSS for Windows, version 18.0; SPSS, Chicago, IL, USA). Data were presented as the mean ± SEM. One-way analysis of variance (ANOVA) with Tukey's post hoc test was used to compare groups of normally distributed data. Nonnormally distributed data were analyzed using the Kruskal-Wallis test. Categorical data were analyzed using the Chi-square test, and Pearson's or Spearman's correlation coefficients were used to test associations between variables. A stepwise multiple regression analysis was performed to identify the predictors of Ang-2 (dependent variable). All tests were two tailed, and values of $P < 0.05$ were considered statistically significant.

Table 1: Clinical and laboratory characteristics.

Group	NC	DN1	DN2	DN3	P
Number	30	38	37	38	—
Age (year)	50.9 ± 2.05	48.21 ± 1.73	51.38 ± 1.73	55.16 ± 1.84	>0.05
Male/female	17/13	23/15	20/17	21/17	>0.05
BMI (kg/m^2)	22.99 ± 0.24	23.13 ± 0.32	23.89 ± 0.33	23.32 ± 0.33	>0.05
SBP (mmHg)	123.2 ± 1.76	119.95 ± 1.14	143.78 ± 1.56	150.11 ± 2.02	<0.001*
DBP (mmHg)	77.47 ± 0.85	80.08 ± 0.77	78.51 ± 0.91	79.92 ± 0.99	>0.05
Total cholesterol (mmol/L)	4.08 ± 0.1	4.47 ± 0.14	4.24 ± 0.15	4.57 ± 0.15	>0.05
LDL-ch (mmol/L)	2.72 ± 0.09	2.7 ± 0.09	2.65 ± 0.09	3.01 ± 0.16	>0.05
TG (mmol/L)	1.71 ± 0.11	1.92 ± 0.08	2.09 ± 0.13	1.8 ± 0.12	>0.05
HDL-ch (mmol/L)	1.26 ± 0.05	1.25 ± 0.04	1.24 ± 0.04	1.42 ± 0.07	>0.05
UAER (μg/min)	0	6.98 ± 0.66	81.31 ± 6.22	338.895 ± 21.92	<0.001*
24 h UPQM (g/24 h)	0	0.099 ± 0.016	0.45 ± 0.03	4.17 ± 0.36	<0.001*
Serum Cr (μmol/L)	67.56 ± 2.43	66.05 ± 1.9	89.18 ± 2.5	236.02 ± 15.89	<0.001*
GFR (mL/min)	87.89 ± 1.31	118.12 ± 2.66	104.07 ± 1.77	49.69 ± 2.84	<0.001*
HbA1c (%)	6.08 ± 0.09	9.02 ± 0.24	9.05 ± 0.27	7.49 ± 0.26	<0.001*
FBS (mmol/L)	5.17 ± 0.05	7.70 ± 0.19	7.5 ± 0.19	6.74 ± 0.22	<0.001*

Data are expressed as mean ± standard error of the mean (SEM). P values were estimated using analysis of variance (ANOVA) or the Kruskal-Wallis test.
NC: normal control; DN1: normal-albuminuria group; DN2: microalbuminuria group; DN3: macroalbuminuria; BMI: body mass index; Cr: creatinine; DBP: diastolic blood pressure; GFR: glomerular filtration rate; FBS: fasting blood sugar; HDL-ch: high-density lipoprotein cholesterol; LDL-ch: low-density lipoprotein cholesterol; SBP: systolic blood pressure; TG: triglyceride; UAER: urinary albumin excretion rate; 24 h UPQM: 24 h urinary protein quantitative measurements; HbA1c: glycosylated hemoglobin; * significant difference between diabetic patients and controls.

3. Results

3.1. Patient Characteristics. The clinical and biochemical characteristics of the study subjects are shown in Table 1. No significant differences were found in age, gender, BMI, diastolic blood pressure (DBP), total cholesterol, LDL-ch, HDL-ch, or TG among the three diabetic groups and the control group. However, systolic blood pressure (SBP), HbA1c, and FBS were higher in the diabetic patients than in control subjects. HbA1c appeared to be lower in the DN3 group than in the DN1 and DN2 groups after long-term treatment with hypoglycemic drugs; however, no significant differences in HbA1c and FBS were found among the three diabetic groups. Serum creatinine was significantly higher, and GFR was significantly lower, in the DN3 group than in the DN1 and DN2 groups ($P < 0.001$). Both UAER and 24 h UPQM increased progressively from the DN1 to the DN3 groups ($P < 0.001$).

3.2. Angiogenic Growth Factors in Serum and Urine. Serum levels of Ang-2 were markedly increased in diabetic patients compared with values in the control group ($P < 0.001$; Figure 1(a)). Moreover, serum Ang-2 was significantly higher in patients with macroalbuminuria (DN3) than those in the DN1 and DN2 groups ($P < 0.001$; Figure 1(a)). Diabetic patients exhibited higher levels of urinary Ang-2 than controls ($P < 0.001$; Figure 1(b)), and urinary Ang-2 increased in a stepwise manner with increasing degrees of albuminuria in the three diabetic groups ($P < 0.001$; Figure 1(b)).

No significant difference was found in serum Ang-1 levels between any of the four groups (data not shown). However, urinary Ang-1 levels were significantly higher in the DN1 group than in the control group ($P < 0.05$; Figure 1(c)) and lower in the DN3 group than in the control group ($P < 0.001$). In addition, patients in the DN1 and DN2 groups had significantly higher urinary Ang-1 levels than those in the DN3 group ($P < 0.001$; Figure 1(c)).

No significant difference was found in serum VEGF among the groups (data not shown); however, subjects with DM exhibited significantly higher urinary VEGF levels than the control subjects ($P < 0.001$; Figure 1(d)). Moreover, urinary VEGF was significantly higher in patients with macroalbuminuria (DN3) than in the DN1 and DN2 groups ($P < 0.001$; Figure 1(d)). No difference was observed in urinary VEGF in diabetic patients with or without microalbuminuria.

3.3. Correlation and Multivariate Analysis. Table 2 and Figure 2 summarize the results of the analyses undertaken in patients with DN. Serum levels of Ang-2 were significantly positively correlated with urinary Ang-2 and VEGF levels (all $P < 0.001$), as well as with UAER, 24 h UPQM (both $P < 0.001$), and serum creatinine ($P < 0.001$). In addition, serum Ang-2 was negatively correlated with GFR ($P < 0.001$).

Similarly, urinary Ang-2 was strongly correlated with urinary VEGF, UAER, 24 h UPQM, and serum creatinine (all $P < 0.001$). A negative correlation was found between GFR and urinary Ang-2 ($P < 0.001$). No significant correlations were found between serum or urinary Ang-2 and age, BMI, DBP, SBP, HbA1c, FBS, HDL-ch, LDL-ch, total cholesterol, or triglyceride (data not shown). In these subjects, stepwise multiple regression analyses including UAER, GFR, age, BMI, DBP, SBP, FBS, HbA1c, and serum Ang-2 identified that UAER ($P < 0.001$) and GFR ($P = 0.001$) are significant predictors of urinary Ang-2.

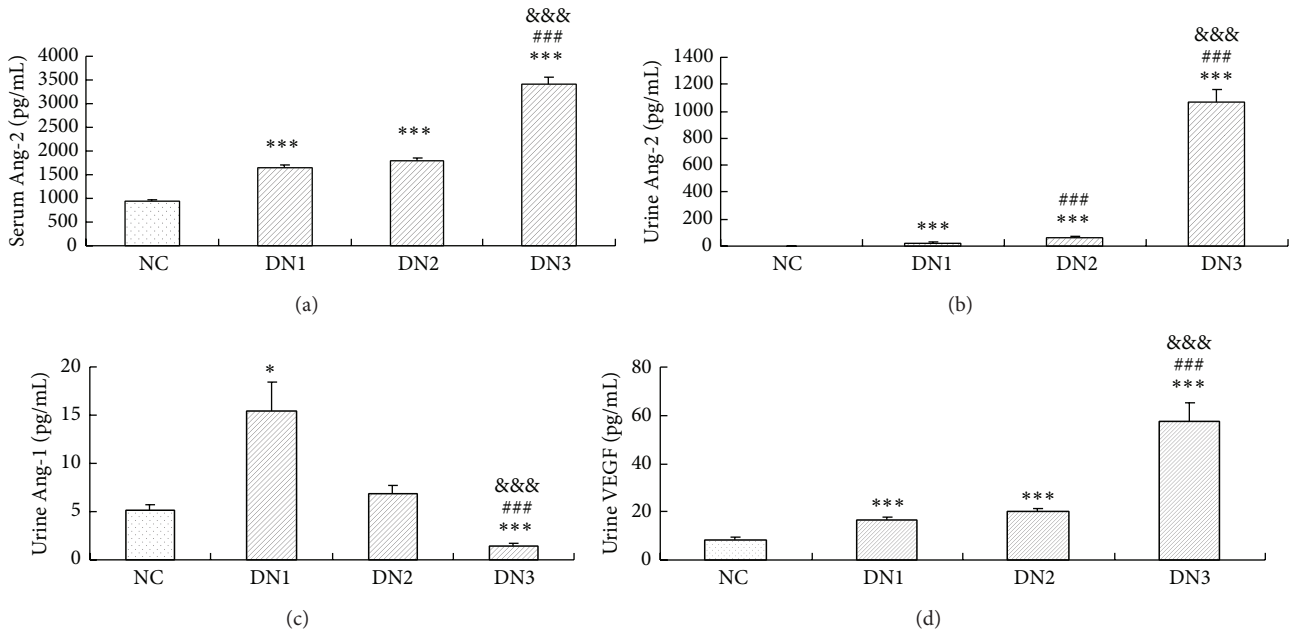

Figure 1: Serum and urinary angiogenic growth factor levels in diabetic patients and controls. (a) Statistical analysis showed increased serum concentrations of Ang-2 in diabetic patients compared with controls. (b) The level of urinary Ang-2 showed a stepwise increase in diabetic patients compared to controls according to the degree of albuminuria. (c) Urinary Ang-1 level was significantly higher in the DN1 group and lower in the DN3 group when compared with control subjects. Patients in the DN1 and DN2 groups exhibited significantly higher urinary Ang-1 levels than those in the DN3 group. (d) Subjects with diabetes mellitus showed significantly higher urinary VEGF levels than control subjects. Patients were divided into DN1 (normal-albuminuria), DN2 (microalbuminuria), and DN3 (macroalbuminuria) groups. $^{*}P < 0.05$ *versus* NC; $^{***}P < 0.001$ *versus* NC; $^{\#\#\#}P < 0.001$ *versus* DN1; $^{\&\&\&}P < 0.001$ *versus* DN2.

Table 2: Correlations between potential markers of DN.

	Urine Ang-2	Serum Ang-1	Urine Ang-1	Serum VEGF	24 h UPQM	GFR	HbA1c
Serum Ang-2	$r = 0.799$ ($P < 0.001$)	$r = -0.022$ ($P > 0.05$)	$r = -0.446$ ($P < 0.001$)	$r = 0.999$ ($P > 0.05$)	$r = 0.826$ ($P < 0.001$)	$r = -0.326$ ($P < 0.001$)	$r = 0.126$ ($P > 0.05$)
Urine Ang-2	—	$r = 0.036$ ($P > 0.05$)	$r = -0.406$ ($P < 0.001$)	$r = 0.112$ ($P > 0.05$)	$r = 0.936$ ($P < 0.001$)	$r = -0.389$ ($P < 0.001$)	$r = 0.17$ ($P > 0.05$)

Data are presented as Pearson's or Spearman's correlation coefficients (r) and P values.
Ang-1: angiopoietin-1; Ang-2: angiopoietin-2; VEGF: vascular endothelial growth factor. For other abbreviations see Table 1.

4. Discussion

This study is the first to investigate urinary levels of Ang-1, Ang-2, and VEGF in human type 2 DM with varying UAERs. We found the following. (1) Urinary Ang-2 increased in a stepwise manner in type 2 DM patients with various degrees of kidney damage (normoalbuminuria, microalbuminuria, and macroalbuminuria). This alteration was accompanied by increased urinary VEGF, as well as early increased and later decreased urinary Ang-1. (2) Urinary Ang-2 levels in normoalbuminuria patients increased prior to changes in albumin levels. (3) Among the angiogenic growth factors, urinary Ang-2 was strongly associated with degree of albuminuria and GFR in type 2 DM patients.

Alterations in the VEGF and Ang-1/Ang-2 system have been reported to play an important role in the pathobiology of glomerular disease in DM [2, 14–17, 20, 26] and chronic kidney disease (CKD) [5, 27–29]. Most of those studies focus on circulating levels of angiogenic growth factors. In this study, we evaluated simultaneously the levels of Ang-1, Ang-2, and VEGF in both serum and urine. We found that urinary Ang-2 increased stepwise with albuminuria levels than Ang-1 and VEGF at a greater rate. In addition, serum and urinary Ang-2 are increased in normoalbuminuric patients. This observation is likely due to the tubular pathophysiological changes, which occur before the glomerular stage of disease. This suggests that the serum and urinary Ang-2 are related to subclinical tubular impairment and may be an earlier measurable marker of renal involvement before the onset of albuminuria.

Increasing evidence suggests that upregulation of Ang-2 is pathologically harmful to the kidney [2]. Ang-2 has been linked to increased microvascular permeability [30], and podocyte overexpression of Ang-2 was shown to produce albuminuria in transgenic mice [22]. Circulating Ang-2 levels correlating positively with proteinuria have been reported in human SLE [21] and CKD [5]. In our type 2 DM patients,

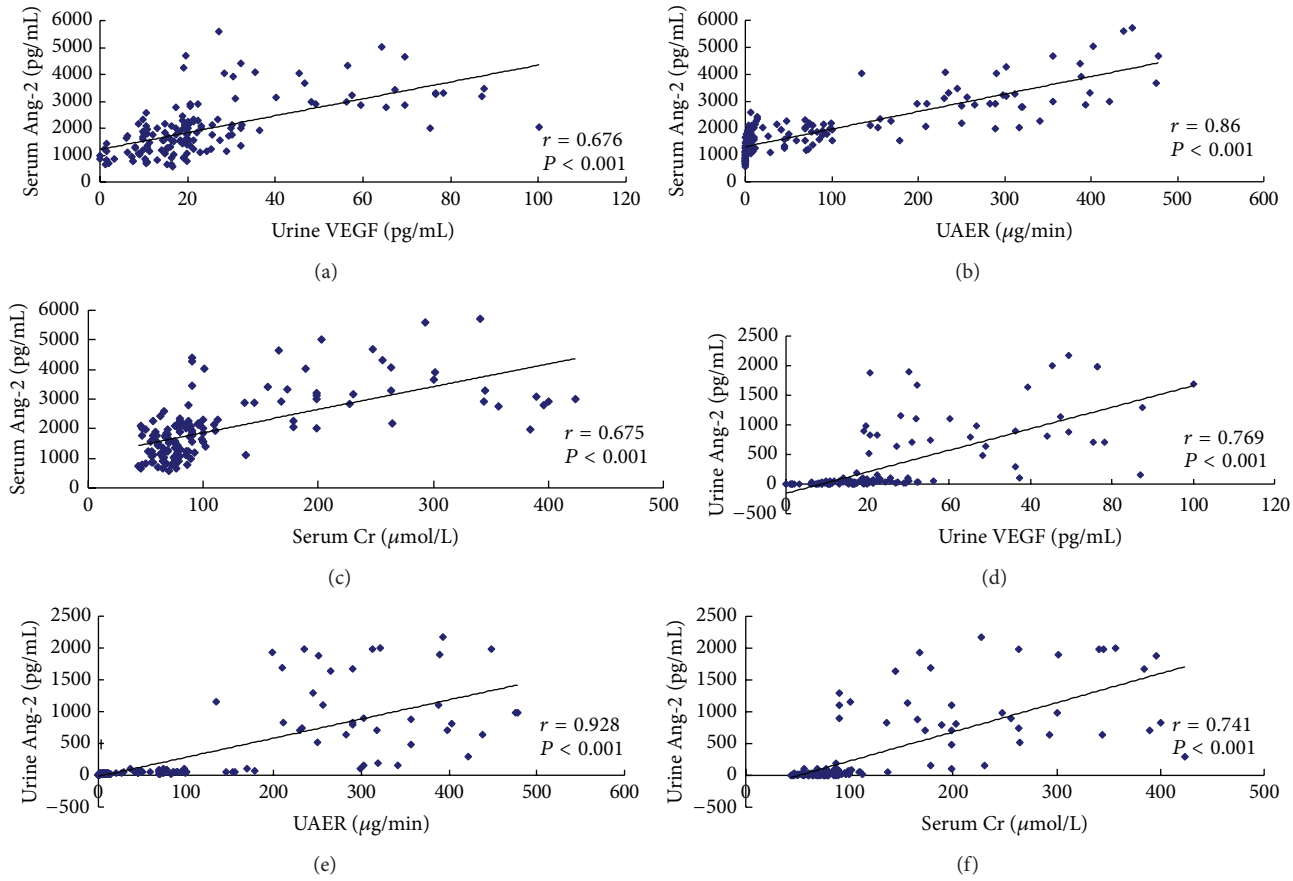

Figure 2: Correlation analysis for serum Ang-2 and urinary Ang-2 with urinary VEGF, UAER, and serum creatinine. Serum Ang-2 level correlated positively with urinary VEGF (a), UAER (b), and serum creatinine (c). Similarly, Urinary Ang-2 correlated positively with urinary VEGF (d), UAER (e), and serum creatinine (f). Ang-2: angiopoietin 2; VEGF: vascular endothelial growth factor; UAER: urinary albumin excretion rate; Cr: creatinine.

we found that albuminuria is a significant predictor for urinary Ang-2 levels after adjustments were made for those possible confounders. The association between urinary Ang-2 and albuminuria suggests that upregulation of Ang-2 may destabilize glomerular endothelial cells and directly or indirectly affect podocytes, leading to the deterioration of glomerular filtration barrier function [5, 31]. Abundant Ang-2 protein was detected by immunohistochemical staining in glomeruli—in endothelial cells alongside capillary loops—in renal biopsies from patients with DM (data not shown). Taken together, these findings suggest that high glucose-induced glomerular endothelial damage may lead to secretion of Ang-2, and elevated expression of Ang-2 in the glomerular endothelium may further increase albuminuria through the damaged glomerular filtration barrier [17]. This possibility requires further investigation.

The positive correlation with serum creatinine and the negative correlation with GFR suggest that increased Ang-2 may be associated with the development of renal impairment. Blood Ang-2 levels rise in line with the decline in renal function in type 2 DM [32] and CKD [29], and this inverse correlation may predict long-term mortality in patients with CKD [27, 33]. In our study, we found that urinary Ang-2 level is inversely related to GFR. These observations suggest that urinary Ang-2 levels increase in parallel to the deterioration of renal function [29]. In our study, elevated urinary Ang-2 was correlated with serum Ang-2, suggesting that urinary levels of Ang-2 may be representative of local production and release of Ang-2 into the circulation in patients with DN. Alternatively, as DN progresses, decreased GFR leads to higher serum Ang-2, which allows greater penetration of the glomerular barrier and leads to proteinuria. However, the multivariate analysis showed that serum Ang-2 is not a predictor for urinary Ang-2. The correlation between serum and urinary Ang-2 requires further study.

Based on the correlation between serum Ang-2 and HbA1c, accumulation of advanced glycation end product (AGE) in endothelial cells subjected to hyperglycemia may upregulate serum levels of Ang-2 and VEGF [34, 35]. However, no correlation was found between urinary and/or serum Ang-2 and HbA1c in our study. The differences between studies may reflect variations in study design. However, the possibility can be not excluded that increased urinary Ang-2 levels are a consequence of mechanisms that are unassociated with the accumulation of AGEs in the glomerular endothelium.

We unexpectedly found that urinary Ang-1 was significantly higher in DM patients with normoalbuminuria and lower in those with macroalbuminuria than in control

subjects. This observation, also reported by Rizkalla, showed upregulation of Ang-1 in the early phase of the disease and progressive downregulation of renal Ang-1 expression in experimental DM [17, 26]. Ang-1 is produced by glomerular podocytes [36, 37] and plays an important role in maintaining the structure and integrity of the glomerular filtration barrier [38]. Increased urinary Ang-1 in DN1 patients compared to control is a novel finding. Further studies will seek to determine whether this increase is a short-term response of podocytes to hyperglycemia or will reduce the vascular permeability through endothelial cell glycocalyx layer [39] modifications or other mechanisms. The decrease in Ang-1 in subjects with macroalbuminuria suggests that Ang-1 production is attenuated at the later stage of DN, which may be associated with a decreased number or function of podocytes, or both. Of course, this possibility requires further investigation. In our study, we found no differences in serum Ang-1 levels in groups with varying degrees of albuminuria. This observation is consistent with previously reported findings [16]. However, Dessapt-Baradez reported decreased Ang-1 levels in mice with streptozotocin-induced type 1 DM, which was accompanied by marked albuminuria, nephromegaly, hyperfiltration, glomerular ultrastructural alterations, and aberrant angiogenesis [38]. The differential expression pattern of Ang-1 in serum may be due to the different subjects and varying stage of DN.

Urinary VEGF was increased in our study in all diabetic groups—even at the normoalbuminuric stage—though serum VEGF was unaltered. These findings are in agreement with previously published findings [10, 14, 18, 40]. However, some centers have reported an increase in plasma VEGF in type 2 DM [19, 41, 42]. These differences likely reflect the different populations being studied, as well as variations in study design.

5. Conclusion

The results of this study show that urinary Ang-2 increases in a stepwise manner in type 2 diabetic patients with varying degrees of kidney damage. Urinary Ang-2 level is associated with albuminuria in type 2 diabetic patients. Ang-2 measurement in urine is a useful, noninvasive tool for the evaluation of renal involvement in the course of DM, especially in normoalbuminuric patients. Further investigations with a larger sample size and a prospective design are required to confirm the potential application of Ang-2 as a useful biomarker for the early detection of diabetic nephropathy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Age- and Gender-Related Differences in LDL-Cholesterol Management in Outpatients with Type 2 Diabetes Mellitus

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Background. Dyslipidemia contribute to the excess of coronary heart disease (CHD) risk observed in women with type 2 diabetes (T2DM). Low density lipoprotein-cholesterol (LDL-C) is the major target for CHD prevention, and T2DM women seem to reach LDL-C targets less frequently than men. *Aim*. To explore age- and gender-related differences in LDL-C management in a large sample of outpatients with T2DM. *Results*. Overall, 415.294 patients (45.3% women) from 236 diabetes centers in Italy were included. Women were older and more obese, with longer diabetes duration, higher total-cholesterol, LDL-C, and HDL-C serum levels compared to men ($P < 0.0001$). Lipid profile was monitored in ~75% of subjects, women being monitored less frequently than men, irrespective of age. More women did not reach the LDL-C target as compared to men, particularly in the subgroup treated with lipid-lowering medications. The between-genders gap in reaching LDL-C targets increased with age and diabetes duration, favouring men in all groups. *Conclusions*. LDL-C management is worst in women with T2DM, who are monitored and reach targets less frequently than T2DM men. Similarly to men, they do not receive medications despite high LDL-C. These gender discrepancies increase with age and diabetes duration, exposing older women to higher CHD risk.

1. Introduction

Type 2 diabetes (T2DM) is a powerful cardiovascular disease (CVD) risk factor in both men and women. Although the overall CVD risk is higher in T2DM men, the relative risk of coronary heart disease (CHD) is higher in T2DM women when compared to nondiabetic ones, with the loss of the typical oestrogen protection in the premenopausal state [1–3].

Although the mechanism underlying this excessive CHD risk in women with type 2 diabetes (T2DM) has not been fully elucidated yet, several hypotheses suggest that diabetes *per se* may be a stronger CHD risk factor in the female gender, determining a more unfavourable CHD risk profile [4, 5]. This could lead to more complex risk factors and/or disease management in women with T2DM as compared to men.

Chronic hyperglycemia may certainly play a role, but it is not the only responsible for the high CHD burden in subjects with T2DM. Thus, cardiovascular disease (CVD) is a multifactorial condition and major risk factors (i.e., obesity, hypertension, and dyslipidemia) have been all demonstrated to contribute to its occurrence [6].

Among these risk factors, low density lipoprotein-cholesterol (LDL-C) is a major target for CVD prevention, also in subjects with T2DM. Thus, the UK Prospective Diabetes Study (UKPDS) demonstrated that LDL-C is the stronger CVD risk factor in subjects with T2DM, even when compared to glycated haemoglobin (HbA1c) and hypertension [7]. Furthermore, several intervention trials with statins have demonstrated the beneficial effect of lowering LDL-C in both primary and secondary CVD prevention, especially in subjects with T2DM [8, 9].

Circulating lipid fractions, including LDL-C, are strongly affected by individual characteristics, including age and gender, but these differences are not always taken into account when managing lipid disorders. In this regard, in a large cohort of Italian T2DM outpatients, we have recently reported that T2DM women were 42% more likely to have LDL-C above the recommended targets, in spite of lipid-lowering treatment and in the context of an overall lower quality of care [10]. Thus, LDL-C levels usually increase with age in men, whereas high density lipoprotein-cholesterol (HDL-C) concentrations tend to decrease with ageing in men, but not in women [11], leading to a ~10 mg/dL between-gender difference in HDL-C levels [12]. Furthermore, gender-related differences have been reported in the pathophysiology, diagnosis, and treatment of lipid disorders.

Since LDL-C is the major goal of CHD prevention in subjects with T2DM, here we further analyzed data on LDL-C management in the large cohort of the AMD Annals initiative [10], in order to assess potential differences according to gender, age, and diabetes duration.

2. Materials and Methods

The population characteristics and study design have been described in detail elsewhere [10]. Briefly, information recorded on electronic medical records between January 1, 2009, and December 31, 2009, of a large sample of patients with a diagnosis of T2DM attending 236 diabetes clinics in Italy was considered. Approximately, one-third of all the Italian diabetes clinics, uniformly distributed throughout the country, were involved in the present study.

The used database derives from the Italian Association of Clinical Diabetologists (Associazione Medici Diabetologi (AMD)) initiative which started in 2006 with the aim of monitoring quality of diabetes care [13, 14]. Its objective was to identify a set of indicators to be used in the context of continuous quality improvement. All participating clinics used an electronic clinical record system for the everyday management of outpatients, and software was specifically developed to extract information from all these clinical databases (AMD data). Data from all participating clinics were collected anonymously and were centrally analysed [13, 14].

The core data set included measures and monitoring of HbA1c, blood pressure, BMI, total-cholesterol, LDL-C or HDL-C, and triglycerides. The use of specific classes of drugs (glucose-lowering, lipid-lowering, and antihypertensive agents), based on ATC codes, was also evaluated.

Current smokers were identified in the electronic clinical record based on a specific Yes/No field. In the case of multiple evaluations of the considered parameters during the year, the most recent ones were taken into consideration for the analysis.

2.1. Statistical Analysis. Clinical characteristics are expressed as mean and standard deviation for continuous variables and frequencies and percentages for categorical ones. The entire sample of studied subjects was divided into two groups according to gender. Further analyses were performed evaluating between-group gender-related differences after stratifying for age, diabetes duration, or both. Some between-gender disparities were also expressed as absolute differences. Even if trivial differences would have reached statistical significance, due to the large sample size, between-group nonparametric statistical tests were applied. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc.).

3. Results

Overall, a total of 415.294 subjects with T2DM followed by 236 diabetes outpatient centers were evaluated. Clinical characteristics of subjects with T2DM included in the analysis according to gender are shown in Table 1. Overall, 45.3% of participants were women. Women with T2DM were older, smoked less, and had a longer diabetes duration and higher BMI, compared with T2DM men ($P < 0.0001$ for all comparisons), whereas they had similar systolic and diastolic blood pressure levels. Women also showed higher HbA1c levels compared to men ($P < 0.001$), except when considering younger subjects (age class <55 years) and a shorter diabetes duration (<2 years) (data not shown). Analysis of lipid profile revealed that women had significantly higher mean total-cholesterol (T-C), LDL-C, and HDL-C serum levels compared to men ($P < 0.0001$ for all comparisons). Serum triglycerides levels were not statistically different between groups. Conversely, LDL/HDL ratio was slightly, but significantly, higher in T2DM men than in women ($P < 0.0001$).

Despite these differences in lipid profile, the use of lipid-lowering medications was overall low and equally distributed in both genders (41.2% of study population). Table 1 shows that, among lipid-lowering drugs, the vast majority of patients (38% of the women and 37% of the men, $P < 0.0001$) were treated with statins; the use of omega 3 fatty acids and fibrates was lower in women than in men ($P < 0.0001$), and very few patients were treated with ezetimibe or bile acids sequestrants in both genders.

As shown in Figure 1, when stratifying study population according to LDL-C targets, a lower percentage of women had LDL-C levels within the recommended values <100 mg/dL as compared to men, whereas the percentage of subjects with LDL-C ≥130 mg/dL was higher in women than in men.

These disparities were also evident when considering patients not taking lipid-lowering medications (subjects with LDL-C <100 mg/dL: 32.0% women versus 37.5% men,

Table 1: Clinical characteristics of T2DM outpatients according to gender.

	Female	Male	*P*
n (%)	188,125 (45.3)	227,169 (54.7)	
Age (yrs)	68.4 ± 11.4	65.7 ± 11.1	<0.0001
Smokers (%)	11.8	21.5	<0.0001
Diabetes duration (yrs)	11.1 ± 9.8	10.0 ± 9.1	<0.0001
BMI (kg/m^2)	30.2 ± 5.9	29.2 ± 4.6	<0.0001
Systolic blood pressure (mmHg)	139.9 ± 19.4	138.6 ± 18.7	<0.0001
Diastolic blood pressure (mmHg)	78.9 ± 9.7	79.3 ± 9.8	<0.0001
HbA1c (mmol/mol)	58 ± 16.4	57 ± 16.4	<0.0001
Total-cholesterol (mg/dL)	194.4 ± 40.9	182.3 ± 40.8	<0.0001
LDL-cholesterol (mg/dL)	112.5 ± 34.8	106.6 ± 34.4	<0.0001
HDL-cholesterol (mg/dL)	53.3 ± 14.0	46.3 ± 12.6	<0.0001
LDL/HDL ratio	2.2 ± 0.9	2.4 ± 1.0	<0.0001
Triglycerides (mg/dL)	143.4 ± 88.3	151.7 ± 121.6	0.40
Patients treated with lipid-lowering medications (%)	41.2	41.2	0.74
Statins use (%)	38.2	37.2	<0.0001
Omega 3 use (%)	1.9	2.4	<0.0001
Fibrates use (%)	3.9	6.5	<0.0001
Ezetimibe use (%)	0.1	0.0	0.22
Bile acids sequestrants use (%)	0.1	0.0	0.12

Data are *n*, %, and means ± standard deviation.

respectively; subjects with LDL-C ≥130 mg/dL: 31.6% women versus 25.7% men, respectively, $P < 0.0001$). Notably, these gender disparities were even more striking in the subgroup of subjects treated with lipid-lowering medications, since the between-genders gap in reaching the LDL-C target of <100 mg/dL was of 7.2% (45.6% versus 52.8% for women and men, resp.), despite the fact that a similar percentage of men and women were treated with lipid-lowering drugs, and a high proportion of women were treated with statins.

3.1. LDL-C Management in T2DM Outpatients according to Gender and Age. Since both age and gender may profoundly affect lipid values, we evaluated several indicators of LDL-C management according to these parameters, stratifying our population in 4 age groups, each with a mean age that was comparable in T2DM men and women (Table 2).

Overall, lipid profile was monitored in about 75% of study subjects, more commonly in the middle age groups (55–75 yrs), whereas those older than 75 years showed the least frequency of monitoring. In each subgroup, women were always monitored less frequently than men, irrespective of age. Furthermore, women did not reach the LDL-C target of <100 mg/dL as men, and this between-genders gap increased with ageing, going from 3.1% in younger subjects to 8.3% in those >75 years old. Accordingly, subjects with higher LDL-C levels (≥130 mg/dL) were more frequent among female participants in all age groups.

3.2. LDL-C Management in T2DM Outpatients according to Gender and Diabetes Duration. Data on LDL-C management were also analyzed according to diabetes duration, which was slightly longer in women than in men (Table 3). Women were 2-3 years older than men in each diabetes duration group. Also, with this stratification, women were less frequently monitored for lipid profile and reached targets less frequently than men. This between-genders gap in favour of men increased along with diabetes duration, going from 5.5% for individuals with a recent diagnosis of diabetes to 7.3% in those with a diabetes duration >10 yrs. Accordingly, when considering the percentages of subjects with a LDL-C ≥130 mg/dL, women showed higher percentages compared with men, across all diabetes duration groups.

3.3. LDL-C Management in T2DM Outpatients according to Gender, Age, and Diabetes Duration. After adjusting for age and diabetes duration (Table 4), LDL-C management was worst in women with T2DM, who were less frequently monitored for lipid profile and less frequently reached LDL-C targets as compared with men. Furthermore, subjects with T2DM of both genders did not receive lipid-lowering medications despite high LDL-C levels in 57.5% of cases.

4. Discussion

Diabetes is a powerful CVD risk factor. Although the absolute risk is higher for men (2.0%) than for women (1.2%), the relative risk of CHD events from having T2DM is higher in women. A meta-analysis of 37 prospective cohort studies showed that the relative risk of fatal coronary heart disease associated with diabetes was higher in women than in men when compared to their nondiabetic counterparts [2]. Thus, the rate of fatal coronary heart disease was substantially higher in people with diabetes than in those without (5.4% versus 1.6%), but this difference, which was apparent in both

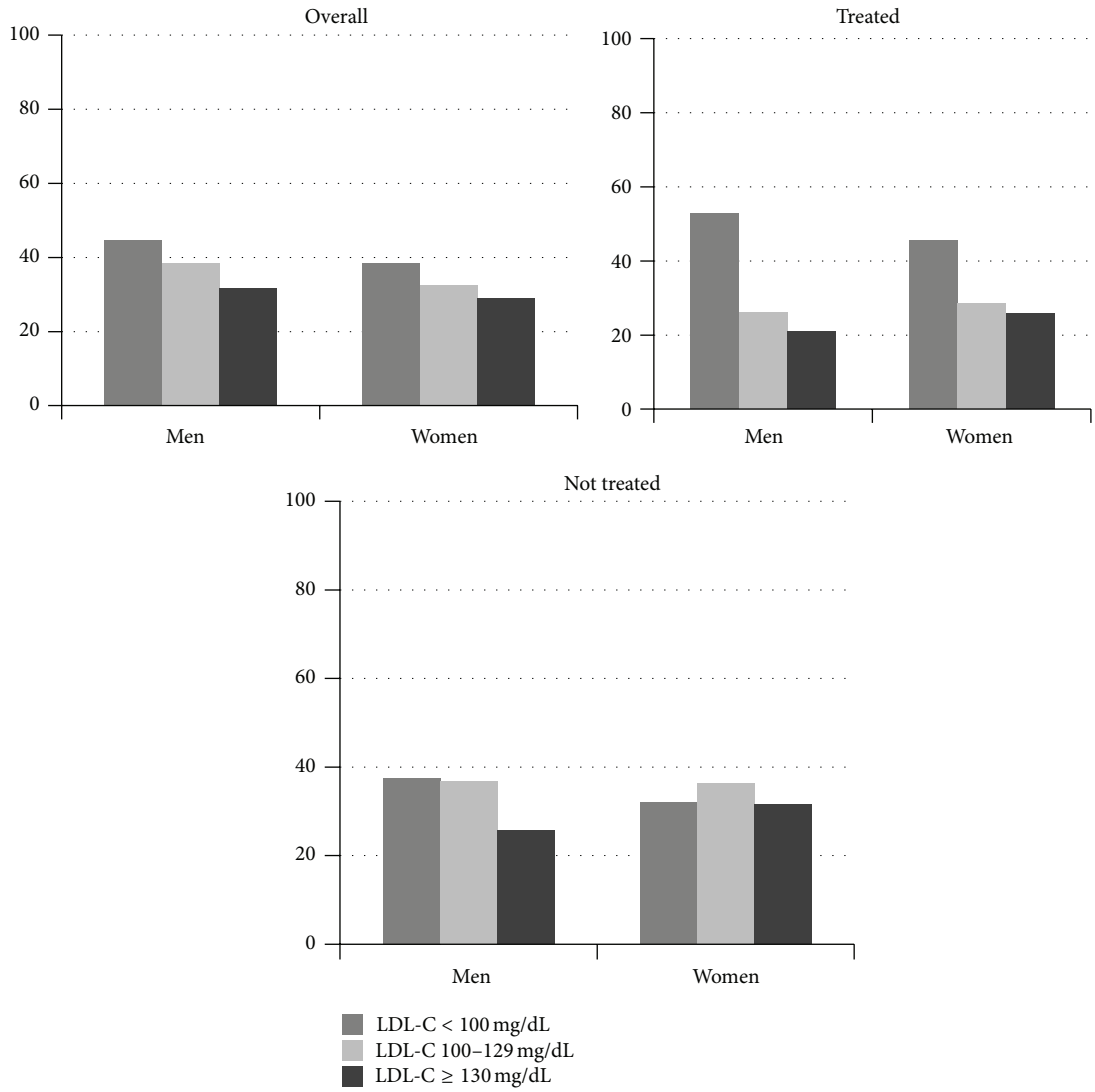

Figure 1: LDL-C classes according to gender and lipid-lowering treatment.

sexes, was more evident among women (7.7 versus 1.2% in those with and without diabetes), whereas the corresponding rates in men were 4.5% and 2.0% [2]. Furthermore, Kalyani and colleagues studied three large cohorts of subjects and found that the risk of incident and fatal CHD in young and middle-aged women with diabetes increased by four- to fivefold as compared to those without diabetes, but these differences were not shown in men [15].

In the recent years, the necessity to consider separately men and women in the process of care was increasingly evident [16]. Gender medicine describes differences in the way of interpreting risk factors, clinical manifestations, and therapeutic approaches in men and women; a better knowledge of these aspects could address prevention and therapeutic strategies, as endorsed by the American Heart Association that recently established specific indications for women [17].

While awaiting an elucidation of the pathophysiological bases of these gender-related differences in CVD, the systematic correction of major modifiable risk factors, that is, a composite control of HbA1c, blood pressure, and LDL-C levels, represents the only strategy to lower CVD risk in patients with T2DM of either sexes.

In a large sample of outpatients with T2DM regularly attending over 200 diabetes units nationwide, we have recently shown that women were more likely to have out-of-target LDL-C levels and an overall poorer quality of diabetes care, as compared to men [10]. Thus, T2DM women were 14% more likely than men to have HbA1c ≥9.0%, 42% more likely to have LDL-C ≥130 mg/dL, and 50% more likely to have BMI ≥30 kg/m^2; they were also less likely to be monitored for foot and eye complications.

Since LDL-C is the major target for CVD primary and secondary prevention, also in subjects with T2DM [12], the

Table 2: Management of LDL-C values in T2DM outpatients according to gender and age.

	<55 years			55–65 years			65–75 years			>75 years		
	F	M	*P*	F	M	*P*	F	M	*P*	F	M	*P*
n	22069	37422		42914	62970		65535	79415		57230	47210	
Age (yrs)	47.1 ± 7.3	47.7 ± 6.4	<0.0001	60.6 ± 2.7	60.4 ± 2.8	<0.0001	70.1 ± 2.8	69.9 ± 2.8	<0.0001	80.6 ± 4.1	79.8 ± 3.7	<0.0001
Diabetes duration (yrs)	6.6 ± 7.2	5.7 ± 6.2	<0.0001	8.6 ± 8.0	8.3 ± 7.5	0.0007	11.4 ± 9.4	11.1 ± 9.2	<0.0001	14.3 ± 11.0	13.9 ± 10.9	<0.0001
Patients monitored for lipid profile (%)	72.2	74.3	<0.0001	75.5	76.4	0.0002	74.9	75.4	0.07	67.3	69.0	<0.0001
Patients with LDL-C <100 mg/dL (%)	32.3	35.4	<0.0001	35.8	43.3	<0.0001	40.6	47.6	<0.0001	40.1	48.4	<0.0001
Patients with LDL-C ≥130 mg/dL (%)	34.9	31.7	<0.0001	31.6	25.4	<0.0001	27.0	20.7	<0.0001	26.8	19.6	<0.0001

Data are *n*, %, and means ± standard deviation.

Table 3: Management of LDL-C values in T2DM outpatients according to gender and diabetes duration.

	<2 years			2–5 years			6–10 years			>10 years		
	F	M	*P*	F	M	*P*	F	M	*P*	F	M	*P*
n	26736	36068		28475	37097		40272	52163		81651	89045	
Age (years)	64.4 ± 12.7	61.4 ± 12.1	<0.0001	65.5 ± 11.7	62.7 ± 11.4	<0.0001	67.2 ± 11.0	64.8 ± 10.5	<0.0001	71.3 ± 10.2	69.1 ± 9.8	<0.0001
Patients monitored for lipid profile (%)	69.8	71.4	<0.0001	74.7	76.4	<0.0001	74.4	75.9	<0.0001	72.4	74.3	<0.0001
Patients with LDL-C <100 mg/dL (%)	28.4	33.9	<0.0001	36.3	41.8	<0.0001	39.2	45.8	<0.0001	42.0	49.3	<0.0001
Patients with LDL-C ≥130 mg/dL (%)	41.6	34.9	<0.0001	30.9	25.6	<0.0001	27.6	22.1	<0.0001	24.9	19.2	<0.0001

Data are *n*, %, and means ± standard deviation.

Table 4: Age-adjusted and diabetes duration-adjusted indicators of quality of LDL-C management according to gender.

	F	M
Patients monitored for lipid profile (%)	73.2	74.4
LDL-C <100 mg/dL (%)	37.8	45.0
LDL-C ≥130 mg/dL (%)	29.2	22.9
Patients with LDL-C ≥130 mg/dL not treated with lipid-lowering medications	57.5	57.5

present study was aimed at investigating how this risk factor is managed in our routine diabetes outpatient activity and whether age, gender, and diabetes duration may influence it.

Although our current paper and that by Rossi et al. [10] consider the same population of subjects with T2DM, they differ in aims, analysis, and outcomes. Rossi and colleagues [10] aimed to evaluate sex differences in pharmacological and nonpharmacological treatment of diabetes and to investigate the role of biological and cultural factors in determining different outcomes for men and women. They performed multilevel regression analyses, taking into account clustering effect, and explored intercenter variability [10]. Conversely, the aim of our present analysis was to focus on potential age- and gender-related differences in lipid management in the routinary care of T2DM outpatients. Therefore, in spite of the simpler statistical analyses used, our study represents a deeper investigation on a particular point the previous paper did not specifically focus on. Our results showed that T2DM women are more likely than men to have LDL-C levels above treatment goals, thus confirming in a larger cohort the results of other studies on this field [2, 18, 19]. This finding was very consistent, since it was shown across subgroups with varying age and duration of T2DM, but also when considering subjects taking or not taking lipid-lowering medications, separately.

The higher LDL-C values in T2DM women not taking lipid-lowering medications may reflect higher basal lipid values in this group, that is, their natural tendency to a worse lipid profile. It is well recognized that lipid profile may vary in women according to age and peculiar biological phases, such as pregnancy and menopause, which are both associated with a more atherogenic lipid profile, with higher total-cholesterol (T-C), LDL-C, and triglycerides concentrations [20–25].

Overall, our data indicate that the gender gap in reaching LDL-C targets increased with ageing and diabetes duration, with older T2DM women (≥75 yrs old) and those with a longer diabetes duration (>10 yrs) showing the greatest (~10%) difference as compared to T2DM men.

Nevertheless, the most striking finding of our study is that T2DM women were not able to reach the recommended LDL-C targets as men, in spite of a similar rate in the use of medications and a slightly higher use of statins. These differences were observed in each age group and in spite of diabetes duration. Several reports have shown gender-related differences in the levels of major CVD risk factors in T2DM cohorts [2, 18, 19] and our data are in agreement with most of these studies, indicating a worse CVD risk profile in T2DM women, despite a similar rate in the use of specific medications. Furthermore, we extended these observations by showing that older women with a longer diabetes duration are those at higher risk of being inappropriately treated. This finding is particularly relevant in the light of the extremely high CHD risk of older T2DM women [26].

Although the cross-sectional nature of our evaluation does not allow us to investigate the causal relationships underlying these gender-related differences in LDL-C management, several possible explanations could be taken into account. Thus, it is likely that, despite similar prescriptions, women are less adherent to treatments [27] or that a treatment bias in favour of men exists. In the latter case, men could receive a better quality of care, with better therapies and, generally, a more complete care [28]. Despite these considerations, it is important here to underline that the burden of CHD is still very high in T2DM men, who are those exposed to the overall higher risk of developing fatal and nonfatal CHD events.

We can also hypothesize that LDL-C levels are different because of a possible different titration of lipid-lowering drugs.

This latter hypothesis would explain some of our results, since women with T2DM in our study were less frequently monitored than men, irrespective of age and diabetes duration. In general, women could also have more social barriers and a lower understanding of the importance of their CVD risk, compared with men. This low perception could be worsened by the presence of stress and lack of time for self-care due to childcare, eldercare, and increasing work activities [29]. All those factors could lead to a lower adherence to treatment in women, as documented in a recent European experience, in which a medication assessment tool was employed to test adherence of patients to evidence-based clinical prescribing recommendations [30]. The other intriguing hypothesis explaining the higher risk of having worse lipid profiles in women compared with men could be a gender-specific lipid-lowering resistance. Sex-specific differences in the pharmacokinetics and pharmacodynamics of drugs are still unclear [27]. Moreover, in the last years, several genes-drugs interactions influencing statins responses were reported [18]. Notably, T2DM men and women may be strikingly different also for lipoprotein subclasses composition and function not limited to LDL-C [31], and, in our study, LDL/HDL ratio was slightly, but significantly, higher in T2DM men than in women. This measure integrates information on both LDL-C and HDL-C, and higher values indicate a higher prevalence of circulating atherogenic particles.

In this regard, another interesting point is the overall low use of nonstatin lipid-lowering medications such as ezetimibe, bile acids sequestrants, omega 3 fatty acids, or fibrates, in the whole cohort and especially in women, who have been reported to be particularly susceptible to the effects of atherogenic dyslipidemia, that is, low HDL-C and high triglycerides levels. Thus, several reports have indicated that CVD risk in T2DM women could depend upon non-LDL-C factors, including atherogenic dyslipidemia or more subtle alterations of lipid profile [31–34]. The modest use of these drugs in women may contribute to the failure to achieve the non-LDL-C lipid targets and to their overall high CVD risk.

5. Conclusions

Our study has several strengths, that is, the large sample size of individuals with T2DM evaluated and the data source utilized. Accordingly, although not comprising all Italian T2DM subjects, our study may be considered a good representation of the quality of diabetes care in Italy. The lack of information on drug doses, sociodemographic and socioeconomic characteristics, and diabetes complications and the impossibility of generalizing our findings to patients cared for by general practitioners may represent limitations of our study.

In conclusion, our data confirm a gender disparity in the routine management of LDL-C levels in T2DM outpatients. Health care professionals should advise women with T2DM about their potential CVD risk, and should not give priority only on treating hyperglycemia and diabetes-related symptoms. Clinicians should also take into account specific gender-related conditions, particularly those capable of influencing CVD risk, with the aim of personalizing their therapeutic actions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Rosaria Cristofaro, and Concetta Suraci were involved in critical revision for intellectual content and interpretation of data. All authors were involved in final approval of the paper to be submitted for publication. Valeria Manicardi is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The authors thank the participating diabetes outpatient centers for their contribution.

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Clinical Study

Sex, Prescribing Practices and Guideline Recommended, Blood Pressure, and LDL Cholesterol Targets at Baseline in the BARI 2D Trial

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Background. Research has shown less aggressive treatment and poorer control of cardiovascular disease (CVD) risk factors in women than men. *Methods.* We analyzed sex differences in pharmacotherapy strategies and attainment of goals for hemoglobin A1c (HbA1c), blood pressure (BP), and low density lipoprotein cholesterol (LDL-C) in patients with type 2 diabetes and established coronary artery disease enrolled into the BARI 2D trial. *Results.* Similar numbers of drugs were prescribed in both women and men. Women were less frequent on metformin or sulfonylurea and more likely to take insulin and to be on higher doses of hydroxymethylglutaryl-CoA reductase inhibitors (statins) than men. After adjusting for baseline differences and treatment prescribed, women were less likely to achieve goals for HbA1c (OR = 0.71, 95% CI 0.57, 0.88) and LDL-C (OR = 0.64, 95% CI 0.53, 0.78). More antihypertensives were prescribed to women, and yet BP ≤ 130/80 mmHg did not differ by sex. *Conclusions.* Women entering the BARI 2D trial were as aggressively treated with drugs as men. Despite equivalent treatment, women less frequently met targets for HbA1c and LDL-C. Our findings suggest that there may be sex differences in response to drug therapies used to treat diabetes, hypertension, and hyperlipidemia.

1. Background

Control of blood glucose, blood pressure (BP), and low-density lipoprotein cholesterol (LDL-C) in patients with type 2 diabetes (DM) and cardiovascular disease (CVD) is key to achieve optimal outcomes [1]. Nationally, attainment of CVD prevention goals for patients with diabetes is suboptimal [2–6] and appears to be worse in women than in men [6–21]. This may be partially explained by a more adverse CVD risk profile in women and/or by differences in therapies given to women compared with men [6, 8, 11, 13, 15, 16, 22–25]. It is often difficult to determine how the dosing of these medications or the class of agents prescribed impact the differences in response to therapies that are seen by sex. Furthermore, less is known regarding whether there are also sex differences in response to drugs used for secondary CVD risk reduction. At present, there are no sex-based differences in guideline treatment recommendations for these three risk factors.

The bypass angioplasty revascularization investigation 2 diabetes (BARI 2D) trial was designed to evaluate outcomes in a cohort of patients with type 2 diabetes and known angiographically documented coronary artery disease (CAD), defined as one or more significant lesions deemed suitable for elective revascularization [26]. The BARI 2D baseline data

set affords an opportunity to compare clinical characteristics and pharmacotherapy prescribing practices in a large cohort of middle-aged men and women with diabetes and CVD recruited 2001–2005. This paper compares the attainment of guideline recommended HbA1c, BP, and LDL-C benchmarks at study entry by sex and the relationship between number, type, and doses of drugs that were prescribed in women and men at study entry. We hypothesized that the approach to drug therapy would be similar in women and men who were enrolled in BARI 2D, and as such benchmark targets for HbA1c, BP, and LDL-C would also be similar by sex after adjusting for the number of relevant drugs prescribed.

2. Methods

BARI 2D (ClinicalTrials.gov Identifier: NCT00006305) is a multicenter, randomized NIH-funded trial designed to determine optimal treatment strategies for patients with DM and documented CAD suitable for elective revascularization. A detailed description of the study design and patient population has been previously reported [26]. Approval was obtained both from the University of Pittsburgh and from individual site institutional committees on human research. Subjects were recruited, consented, and randomized from 49 clinical sites in USA, Canada, Brazil, Mexico, the Czech Republic, and Austria between January 2001 and March 2005. Eligibility criteria included a diagnosis of DM and angiographically documented CAD not requiring immediate revascularization.

At the time of randomization, demographics, clinical history, physical exam, test results, and medications were collected. HbA1c and lipids were measured in a BARI 2D core laboratory and secondarily at point of care for clinical management decisions. Only those patients with quality baseline information were included in the present analysis. To classify level of control for study-designated treatment targets, measures of HbA1c, fasting LDL-C, and BP were collected. United States guideline recommendations for treatment goals for diabetes, hypertension, and cholesterol were set at <7% for HbA1c, <100 mg/dL for LDL-C, and ≤130/80 mm Hg for BP during the BARI 2D recruitment years [27] until 2004 when the LDL-C goal was tightened to allow consideration of <70 mg/dL [28]. Core laboratory derived HbA1c and LDL-C were available in 95% and 92% of patients, respectively. Missing core lab values were augmented by clinical site measures.

Therapeutic agents were categorized into antianginal/antihypertensive, antiplatelet/anticoagulant, antihyperlipidemic, and antidiabetes agents. Antidiabetes drugs were further subdivided into insulin providing (IP), insulin sensitizing (IS), and IP-IS neutral [29]. Each drug and its total daily dose at study entry were recorded. Diabetes agent and statin doses were further substratified to designate their being either within or above recommended starting dose(s) as stated in FDA approved prescribing information as of September 2007. The latter analysis was not performed for BP lowering drugs as these medications were not solely prescribed for BP control and we were not able to ascertain the indication (s) for which each BP agent was prescribed.

Statistical comparisons of proportions and means were made between sexes for demographic variables, clinical history, lab measures, and use of pharmacotherapeutic agents. For lipid lowering and oral diabetes agents, the proportion of patients whose clinical measures were at target was also compared by sex according to dose stratification. Chi-square tests and t-tests were performed as appropriate; P values less than 0.05 were considered statistically significant. In order to test sex differences in achieving treatment goals, outcomes of multiple logistic regression models for the defined targets of HbA1c and LDL-C BP were evaluated. Odds ratios of achieving treatment targets for women and men were calculated using logistic models adjusted for age, race ethnicity, education, physical activity, current cigarette smoking status, BMI, duration of diabetes, history of CAD prior to enrollment, hypertension, and number of relevant medications. All analyses were performed using SAS version 9.1.3 (Cary, NC).

3. Results

Among the 2368 patients enrolled, 2321 subjects had quality data and were included in the analysis. Among this group, there were 686 women, (mean age 62.9 years, 44.5% nonwhite) and 1635 men (mean age 62.2 years; 30.2% nonwhite). Demographic and clinical history characteristics are shown in Table 1. Women entering BARI 2D had a heavier burden of CVD risk factors than men, including higher BMI, longer duration of diabetes, higher prevalence of hypertension, a more sedentary lifestyle, and worse self-related health. Women less often had a history of cigarette smoking and were less likely than men to have had prior MI or CABG. Women had higher HbA1c and LDL-C levels and higher average BP than men.

Table 2 depicts the pharmacotherapeutic agents that were prescribed for women and men just prior to study entry. A similar percentage by sex was treated with most categories of agents, although significantly fewer women were taking metformin and sulfonylureas. Insulin and diuretics were being taken by more women than men. A similar number of women and men were taking some form of antiplatelet/anticoagulant; however, fewer women than men were taking aspirin.

The average number of drugs prescribed for each category of risk was determined. Within the category of antidiabetes agents, the number of drugs being taken did not differ between women and men (1.54 ± 0.81 versus 1.58 ± 0.88, $P = 0.30$). However, women were taking more antihypertensive drugs (2.36 ± 1.05 versus 2.17 ± 1.01, $P < 0.001$) and fewer lipid lowering drugs (0.84 ± 0.53 versus 0.91 ± 0.58, $P = 0.004$) than men. The average number of drugs being taken for the 4 risk categories assessed, including antiplatelet/anticoagulants, approached 7 agents and did not differ between women and men (6.64 ± 2.08 versus 6.57 ± 2.17, $P = 0.43$).

The average drug dose and the percentage of patients on titrated doses for statins and antidiabetes agents are shown in

Table 1: BARI 2D demographics and clinical history by sex.

Demographic/clinical history	Female ($N = 686$)	Male ($N = 1635$)	P value
Age at entry (years), mean ± SD	62.9 ± 9.3	62.2 ± 8.7	0.08
Race ethnicity			***<0.001***
White, non-Hispanic	55.5%	69.8%	
Black or African-American, non-Hispanic	27.7%	12.4%	
Hispanic	13.0%	12.5%	
Asian and others	3.8%	5.3%	
Region and country			***<0.001***
United States and Canada	74.9%	79.1%	
México and Brazil	22.1%	17.7%	
Czech Republic& Austria	3.1%	3.3%	
Education level			***<0.001***
<High school	46.1%	33.0%	
≥High school	53.9%	67.0%	
BMI (kg/m^2), mean ± SD	32.9 ± 6.9	31.3 ± 5.4	***<0.001***
Diabetes duration (years), mean ± SD	12.2 ± 9.6	9.7 ± 8.1	***<0.001***
History of hypertension	87.2%	80.4%	***<0.001***
History of hypercholesterolemia	82.9%	81.4%	0.4
History of MI	27.9%	33.6%	***0.007***
History of CHF	7.8%	6.0%	0.1
CABG prior to randomization	4.5%	7.2%	***0.02***
PCI prior to randomization	20.1%	19.4%	0.7
Cerebrovascular Accident	11.0%	9.3%	0.2
Physical Activity			***<0.001***
Sedentary	28.2%	19.3%	
Mild to moderate	69.6%	77.4%	
Strenuous	2.2%	3.3%	
Cigarette smoking			***<0.001***
Never smoked	51.9%	25.4%	
Current smoker	9.8%	13.4%	
Self-rated health			***<0.001***
Excellent to good	46.8%	56.4%	
Fair to poor	53.3%	43.6%	
Mean HbA1C (%)	8.0 ± 1.7	7.5 ± 1.6	***<0.001***
Mean blood pressure (mm Hg)			
Systolic (mm Hg)	134.8 ± 22.6	130.4 ± 18.7	***<0.001***
Diastolic (mm Hg)	73.8 ± 12.5	74.9 ± 10.6	***0.04***
Mean LDL cholesterol (mg/dL)	102.9 ± 34.8	93.5 ± 32.2	***<0.001***

MI = myocardial infarction; CHF = congestive heart failure.

Table 3. Women were taking higher average doses of atorvastatin and pravastatin than men. For each antidiabetes drug, average doses did not differ and the percentages on titrated doses were similar by sex, except for the percentage on >0.4 units/kg/day of insulin which was being taken by more women. There were no significant differences in the percent of women and men taking statin dose above the recommended starting dose(s) per FDA approved prescribing information.

An analysis by sex of the percent of subjects who met the prespecified clinical targets for HbA1c, BP, LDL-C, and BP is detailed in Table 4. Significantly, fewer women were at goal for both HbA1c and LDL-C than men. There was no difference by sex in the percent at target for BP. After adjustment for covariates, including clinical variables as well as number of relevant agents prescribed, the odds of being at target for HbA1c and LDL-C remained significantly lower for women than for men. The odds of being at target for BP remained similar.

4. Discussion

Control of CVD risk factors substantially improves outcome among high-risk patients with DM [1]. Based on this information, clinical guidelines specific to individuals with diabetes that were in effect at the time of BARI 2D recruitment specified benchmark targets for control of HbA1c, LDL-C, and BP. The BARI 2D baseline data analysis allows comparison of physician prescribing practices, intensity of drug therapy prescribed, and degree of attainment of standards of care for HbA1c, LDL-C, and BP in women and men with DM

Table 2: Pharmacotherapeutic agents by target category and by class.

Pharmacotherapeutic agent	Total (N = 2321)	Female (N = 686)	Male (N = 1635)	P value
Antidiabetes agents				
Any diabetes drug	91.4%	92.1%	91.1%	0.44
Insulin sensitizing	60.9%	57.7%	62.3%	***0.04***
Metformin	54.1%	50.2%	55.7%	***0.02***
TZD	18.8%	16.4%	19.8%	0.06
Insulin Providing	75.6%	77.8%	74.7%	0.11
Sulfonylurea	53.6%	47.7%	56.0%	***<0.001***
Meglitinide	0.7%	1.0%	0.6%	0.29
Insulin	27.8%	36.3%	24.3%	***<0.001***
Lipid lowering agents				
Any lipid drug	79.1%	77.3%	79.9%	0.16
Statin	74.7%	73.0%	75.4%	0.22
Fibrate	8.6%	6.3%	9.6%	***0.01***
Niacin	2.2%	1.5%	2.4%	0.14
Blood pressure agents				
Any blood pressure drug	95.8%	95.8%	95.8%	1.00
ACE or ARB	77.1%	75.6%	77.7%	0.27
Beta-blocker	72.9%	74.3%	72.3%	0.32
Calcium channel blocker	31.4%	33.9%	30.4%	0.10
Diuretic	38.7%	49.9%	34.0%	***<0.001***
Antiplatelet/anticoagulants				
Any antiplatelet/anticoagulant	91.9%	90.5%	92.5%	0.10
Aspirin	88.0%	85.7%	89.0%	***0.03***
Ticlopidine/clopidogrel	18.0%	18.8%	17.6%	0.49

ACE = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker.

Table 3: Average daily drug dose and percentage on titrated doses for diabetes agents and statins by sex.

Agent	N		Average daily dose (mg)			% on titrated dose			
	Female	Male	Female	Male	P value	Threshold	Female	Male	P value
Taking diabetes drug	**622**	**1465**	—	—	—	Diabetes drugs	**72%**	**73%**	**0.63**
Any insulin sensitizing	**394**	**1015**	—	—	—	IS	**58%**	**62%**	**0.27**
TZD	111	322	—	—	—	TZD	46%	42%	0.46
Pioglitazone	61	139	32.5	31.3	0.49	>30 mg	36%	32%	0.54
Rosiglitazone	50	183	6.7	6.0	0.12	>4 mg	58%	50%	0.30
Metformin	344	907	1517.5	1526.2	0.83	>1000 mg	58%	61%	0.31
Any insulin providing	**515**	**1162**	—	—	—	IP	**71%**	**69%**	**0.34**
Sulfonylurea	316	864	—	—	—	SU	63%	64%	0.66
Glyburide	192	540	18.0	13.5	0.30	>5 mg	75%	73%	0.58
Glipizide	87	236	17.6	14.0	0.31	>10 mg	31%	44%	***0.03***
Glimepiride	37	88	5.1	4.3	0.17	>2 mg	76%	66%	0.28
Insulin	249	397	0.7 unit/kg	0.6 unit/kg	0.17	>0.4 unit/kg	75%	68%	***0.04***
Taking statin	**483**	**1186**	—	—	—	Statins	**46%**	**46%**	**0.96**
Atorvastatin	181	443	29.4	25.0	***0.01***	>10 mg	66%	62%	0.36
Lovastatin	16	42	28.8	35.5	0.18	>20 mg	38%	64%	0.07
Pravastatin	41	92	32.9	24.8	***0.002***	>40 mg	5%	1%	0.17
Simvastatin	245	609	28.8	29.8	0.53	>20 mg	39%	41%	0.71

IS = insulin sensitizing; IP = insulin providing diabetes drugs; SU = sulfonylurea.

Table 4: Achievement of clinical targets for HbA1c, blood pressure, and LDL-cholesterol at baseline in BARI 2D by sex.

Clinical target	% at clinical target			Odds ratio (95% CI)†	
	Female ($N = 686$)	Male ($N = 1635$)	P value	Unadjusted female versus male	Adjusted† female versus male
HbA1c < 7%	31.9%	42.3%	***<0.001***	0.64 (0.53, 0.77)	0.71 (0.57, 0.88)
Blood pressure ≤ 130/80 mm Hg	45.0%	48.6%	0.12	0.87 (0.72, 1.04)	1.11 (0.92, 1.35)
LDL < 100 mg/dL	49.8%	63.1%	***<0.001***	0.58 (0.48, 0.69)	0.64 (0.53, 0.78)
Achieved all 3 target goals	10.9%	15.8%	***0.002***	0.65 (0.49, 0.86)	0.78 (0.58, 1.04)

†All clinical targets were adjusted for the following common covariates: sex, age, race ethnicity, education, physical activity, cigarette smoking, duration of diabetes, and BMI. In addition, HbA1c was adjusted for a number of diabetes agents; lipids targets were adjusted for a number of lipids agents and CABG or PCI prior to randomization; blood pressure target was adjusted for a number of antihypertensive agents, CABG or PCI prior to randomization, and history of hypertension. The attainment on all 3 targets was controlled for a number of total drugs, CABG or PCI prior to randomization, and history of hypertension.

and established CAD across a diversity of physician practice settings. The findings of these baseline data suggest that women enrolled in the BARI 2D trial were as intensively treated with drugs for DM and CVD prevention as men at study entry, with the exception of aspirin which was taken by fewer women than men. Despite equivalence in prescribing practices, women met benchmark targets for HbA1c and LDL-C less often than men. The adjusted odds ratio was in the same direction and of similar magnitude for HbA1c and LDL-C, compared with the unadjusted odds. This demonstrates a robust relationship between sex and achievement of targets. Our findings are consistent with some prior reports that demonstrated that women are less likely than men to achieve control of HbA1c [6, 8, 11, 17, 18] and LDL-C [6–11, 17–21]. A few studies have reported on the likelihood of achieving guideline targets after adjusting for the type of drugs prescribed [11, 12, 30–32]. In the recent report by Rossi et al., of a cohort of Italian diabetes clinic patients, inequalities in attainment of benchmarks were observed with women being more likely than men to have A1C >9.0% in spite of insulin treatment and to have LDL >130 mg/dL in spite of lipid lowering treatment [31], as was the case in the BARI2D cohort. Both the BARI2D and the Italian cohorts demonstrated medical undertreatment of women with aspirin and in the latter undertreatment with ACE-inhibitors was also observed among females. In contrast to these findings, among a Swedish cohort of adults with chest pain referred for a first time diagnostic elective coronary angiography from 2006 to 2008, it was shown that female sex was independently associated with underutilization of guideline recommended therapy. The Swedish data revealed subsequent equivalent use of ACE-inhibitors, beta-blockers, aspirin, and statins among women and men after angiographic diagnosis of obstructive CAD was made [32].

Our detailed analysis of pharmacotherapeutic agents used to control HbA1c, LDL-C, and BP among patients entering the BARI2D study demonstrates that, despite a similar intensity of medications used to control CVD risk factors, women were still less likely to achieve target goals for HbA1c and LDL-C than men. Women enrolled in the BARI 2D study had a less favorable risk profile at baseline compared with men, including greater age, longer duration of DM, higher BMI, a higher prevalence of hypertension, a more sedentary lifestyle, and a lower level of education. Although these variables might affect the ability to control HbA1c, LDL-C, and BP, differences were still noted among women and men even after adjustment for these variables, suggesting that alternate factors are likely at play in this sex gap. Various other explanations for the observation that women are less likely than men to reach treatment goals for HbA1c, LDL-C, and BP have been put forward, including biologic factors, medication adherence, and possible differences in the quality of health care delivery by sex.

Women have been shown to receive less aggressive therapies to treat or prevent CVD than men [6, 8, 11, 13, 15, 16, 22–25, 31–33]. In the current report, however, we performed a detailed analysis of number and intensity of medications prescribed for women and men and found very similar dosing of medications used to treat CVD risk factors. If anything, the intensity of therapy was greater in women than men. More women than men were treated with insulin in keeping with their longer duration of diabetes and higher HbA1c levels. Insulin doses (units/kg body weight/day) were also higher in women. The findings that more women than men in BARI 2D were treated with insulin and that women were taking a higher number of units of insulin/kg of body weight daily when compared to men suggest the possibility of a greater degree of insulin resistance among the women. This possibility is also supported by the presence of a higher BMI and a more sedentary lifestyle among the women subjects. Statins were prescribed to a similar percentage of women and men, and a similar number by sex were on a "titrated" dose of statin. The average dose of statins tended to be higher in women. Although fewer women than men were treated with fibrate therapy, the total number of drugs used to treat hypercholesterolemia was similar by sex. On average more total antihypertensive drugs were prescribed to women than men. A similar percentage of women and men were taking an ACE-inhibitor or ARB and/or beta-blockers and more women than men were taking diuretics. The only CVD prevention agent which was prescribed less frequently among BARI2D women was aspirin. Given these findings, we do

not feel that the differences reported were a result of sex differences in prescribing practices.

It is possible that there are inherent biological differences by sex in the response to the pharmacotherapeutic agents used to treat CVD. For example, studies have shown sex differences in the biologic and clinical response to antiplatelet drugs [34, 35], while other studies have suggested differences in the time to achieve adequate control of LDL-C as a function of race and sex [30]. Differences in response to therapies may relate to sex differences in enzymatic activities, glomerular filtration, levels of endogenous hormones, body surface area, and proportion of body fat. It is possible that these biologic differences in women and men impact the efficacy of the drugs used to treat DM, high cholesterol, and hypertension. Previous reports have demonstrated poorer compliance with medications and lifestyle interventions in women than men [7, 20, 36] and an association of adherence to medications and achievement of target goals [7]. Compliance with medications can be influenced by the cost of the medication, the patient's underlying condition, the frequency of follow-up visits, the use of mail order pharmacies, and sociodemographic variables [20]. Information regarding adherence to therapy prescribed and dietary and lifestyle practices at study entry in BARI 2D was not recorded. We did, however, show that women enrolled in BARI 2D were older than men, had a higher BMI, led more sedentary lifestyles, and had poorer education; all of these factors may directly impact adherence. Our multivariate model attempted to adjust for these variables; however, even after adjustment, sex differences in achievement of goals persisted.

It is also possible that the differences in the outcomes reported by sex relate to higher pretreatment levels of HbA1c, LDL-C, and BP. Some studies have demonstrated that the ability to adequately lower LDL-C is directly correlated with starting LDL-C values [37]. Since we did not have information regarding the "pretreatment" indices for HbA1c, LDL-C, and BP, we cannot determine whether these parameters had any effect on achievement of benchmarks for these targets.

5. Limitations

Baseline data presented in this analysis were obtained by each subject's self-report. Therefore, information on drugs previously prescribed, their side effects, and information on subject adherence with the prescribed therapy were not available. Each of these variables could potentially impact sex differences in target attainment and limits our ability to determine whether the differential attainment of targets between the sexes is due to adherence factors or actual response to therapy. In addition, the recruitment of subjects from academic medical centers may bias the data, limiting the ability to extrapolate findings to community practices.

6. Conclusions

Women enrolling in BARI 2D were being treated as intensively with diabetes, lipid lowering, and blood pressure drugs as men. Despite equivalence of therapies prescribed, including number of agents prescribed and an apparently equivalent degree of drug dose titration, women less frequently met targets for HbA1c and LDL-C than men. These findings suggest that sex differences in attaining clinical targets cannot be explained solely by sex bias in drug prescribing practices. Other variables such as differences in medication adherence or differences in therapeutic responses to agents used for secondary CVD prevention among women compared to men must be considered. As we strive to decrease the percentage of both women and men with type 2 diabetes who die from CVD, further studies are needed to investigate sex-specific factors that may impact targeted management of risk factors for CVD.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Differences in the Cardiometabolic Control in Type 2 Diabetes according to Gender and the Presence of Cardiovascular Disease: Results from the eControl Study

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The objective of this cross-sectional study was to assess differences in the control and treatment of modifiable cardiovascular risk factors (CVRF: HbA1c, blood pressure [BP], LDL-cholesterol, body mass index, and smoking habit) according to gender and the presence of cardiovascular disease (CVD) in patients with type 2 diabetes mellitus (T2DM) in Catalonia, Spain. The study included available data from electronic medical records for a total of 286,791 patients. After controlling for sex, age, diabetes duration, and treatment received, both men and women with prior CVD had worse cardiometabolic control than patients without previous CVD; women with prior CVD had worse overall control of CVRFs than men except for smoking; and women without prior CVD were only better than men at controlling smoking and BP, with no significant differences in glycemic control. Finally, although the proportion of women treated with lipid-lowering medications was similar to (with prior CVD) or even higher (without CVD) than men, LDL-cholesterol levels were remarkably uncontrolled in both women with and women without CVD. The results stress the need to implement measures to better prevent and treat CVRF in the subgroup of diabetic women, specifically with more intensive statin treatment in those with CVD.

1. Introduction

The prevalence rates of diabetes mellitus (DM) have significantly increased during the last years, accompanied by a parallel rise in complications and deaths from the disease [1, 2]. The worldwide prevalence in 2013 has been estimated to be 8.3%, and it is expected to be about 1 adult in 10 by 2035, which represents a substantial 55% increase [3, 4]. A recent population-based survey conducted in Spain reported a global prevalence of DM of 13.8% in adult subjects, and 43.5% of them were unaware of their disease, thus corresponding to a prevalence of unknown DM of 6% [5].

People with type 2 diabetes mellitus (T2DM) are at increased risk of cardiovascular complications such as coronary artery disease, stroke, or peripheral vascular disease [6, 7]. In turn, these complications are associated with increased

morbidity and mortality and have a detrimental effect on health-related quality of life [8]. Current available evidence indicates that, in terms of risk reduction of cardiovascular and microvascular complications, control of blood pressure and lipid levels is more effective than glucose control [9–11]. Additionally, type 2 diabetic patients with clinical cardiovascular disease (CVD) are at a higher risk of a recurrent cardiovascular event [12, 13]. However, several studies have shown that, in clinical practice, secondary prevention strategies in diabetic patients with CVD are associated with a suboptimal cardiometabolic control [11, 14].

Systematic reviews of the literature have reported that the excess relative risk of CVD attributable to diabetes is 2-fold in men and 3- to 4-fold in women [15, 16], and this has been further confirmed by several meta-analyses [17–19]. Some authors have postulated that diabetes prompts the loss of the natural hormonal protection against CVD in women [20, 21], but several factors that may explain this excess risk in women relative to men have been identified so far [22–25], and they mainly include a low risk perception by health care providers [26]; an increased time to proper medical care from the onset of symptoms; a lower predictive capability of certain diagnostic tests (e.g., stress test); a differential drug response among women to some medications such as aspirin [27] or statins [28], which decreases their effectiveness; and worse clinical outcomes after therapeutic procedures [29].

Cross-sectional studies have reported that the control of cardiovascular risk factors (CVRF) is poorer among diabetic women relative to diabetic men of the same age [30–32]. Moreover, the follow-up of the population in the National Health and Nutrition Examination Surveys (NHANES) has shown that, for the past years, there has been an overall decline in mortality rates due to CVD, but not in the subgroup of diabetic women [33].

On the other hand, studies derived from the analysis of large databases have proven to be useful for evaluating cardiometabolic control, associated risk factors, long-term complications, and other clinically relevant aspects of T2DM [34–37].

The aim of the present population-based study was to assess differences in the degree of control and treatment of modifiable CVRF according to gender and CVD in patients with T2DM in Catalonia, Spain.

2. Materials and Methods

2.1. Design. This cross-sectional study includes all type 2 diabetes subjects visiting any of the 274 primary care centres pertaining to the Catalan Health Institute (ICS) in Catalonia, a northeastern region of Spain, which takes care of a population of about 5.8 million patients (80% of the total population for the region).

The data for the present study (eCONTROL) were extracted from SIDIAP (Information System for the Development of Research in Primary Care; SIDIAP) [38], a database of electronic medical records started in 2006. Methodological details of the study of diabetes mellitus using this database have been described in previous publications [36, 39]. Briefly, SIDIAP contains anonymized longitudinal patient information obtained through use of specific software (eCAP) implemented in all primary care centers in Catalonia and includes sociodemographic characteristics, morbidity (by means of International Classification of Diseases codes; ICD-10), clinical and lifestyle variables, specialist referrals, and results of laboratory tests and treatments based on prescription- and pharmacy-invoicing data provided by the CatSalut general database.

2.2. Data Extraction. Data from patients attended before July 1, 2009, aging 31 to 90 years, and with a diagnosis of type 2 diabetes (ICD-10 codes E11 or E14) were extracted from the SIDIAP database [36]. Available variables (registered up to the end of 2009) included age; gender; time since diagnosis; estimated glomerular filtration rate (eGFR) using the Modified Diet in Renal Disease (MDRD) formula; standardized glycated haemoglobin (HbA1c) values, using the most recent value of the preceding 15 months; presence of cardiovascular disease, including coronary artery disease (ICD-10 codes I20, I21, I22, I23, or I24), stroke (ICD-10 codes I63, I64, G45, or G46), and peripheral artery disease (ICD-10 code I73.9); risk factors, including body mass index (BMI) (most recent value in the last 24 months), cholesterol levels (total, low-density lipoproteins or LDL-cholesterol, and high-density lipoproteins or HDL-cholesterol; most recent value in the last 15 months), blood pressure (BP) (systolic and diastolic mean value in the last 12 months), smoking status (most recent value); and data on prescribed glucose-lowering, lipid-lowering, and antihypertensive and antithrombotic medications.

Diagnostic criteria for CVRF were HbA1c > 7%; hypertension (blood pressure > 140/90 mmHg); hypercholesterolemia (total cholesterol > 250 mg/dL); hypertriglyceridemia (triglycerides > 150 mg/dL); obesity (BMI > 30 kg/m^2); and current or former smoking habit. Treatment goals for patients with and without a history of CVD were based on local guidelines [40, 41]; without CVD prevention: HbA1c ≤ 7%, BP ≤ 140/90 mmHg, and LDL-cholesterol ≤ 130 mg/dL; with CVD prevention: HbA1c ≤ 7%, BP ≤ 140/90 mmHg, and LDL-cholesterol ≤ 100 mg/dL.

This study was approved by the Ethics Committee of the Primary Health Care University Research Institute (IDIAP) Jordi Gol.

2.3. Statistical Analysis. Descriptive analyses were summarized by mean and standard deviation for continuous variables and percentages for categorical variables. Comparisons by gender and presence of CVD were performed with Pearson chi-square tests for categorical variables and analysis of variance (ANOVA) for continuous variables. We applied multilevel logistic regression models to identify the factors associated with good cardiometabolic control of CVRFs. Only those variables with a statistically significant effect ($P < 0.05$) in the univariate analyses were retained for the multivariate model. Analyses were performed stratifying according to the presence of CVD, and odds ratios (OR) and 95% confidence intervals (95% CI) were adjusted for gender, diabetes duration, and treatment as confounding variables. Statistical calculations were performed using StataCorp 2009

(Stata Statistical Software: Release 11. College Station, TX: StataCorp, LP).

3. Results

The study included data from a total of 286,791 patients with T2DM (153,987 men and 132,804 women). Overall, 18.4% of the patients ($N = 52{,}665$) had a previous history of any CVD, which was more frequently reported among men (22.3% versus 13.8%).

In the overall population, all studied variables showed significant differences between men and women; women were in average older than men, had a longer duration of the disease, and had slightly worse cardiometabolic control than men, with higher blood pressure levels (mean 137.5/76.2 mmHg versus 136.9/76.6 mmHg; $P < 0.005$), higher LDL-cholesterol levels (mean 115.6 mg/dL versus 109.7 mg/dL; $P < 0.005$), and higher average BMI (mean 30.5 versus 28.8; $P < 0.005$), but slightly better control of the percentage of HbA1c than men (7.1% versus 7.2%; $P < 0.005$) (Table 1). Moreover, triglyceride levels were lower in women (mean 153.5 mg/dL versus 158.5 mg/dL; $P < 0.005$), and there were far more nonsmokers among diabetic women (88% versus 43.5%; $P < 0.005$).

3.1. Cardiometabolic Control of T2DM and Degree of Control of CVRF according to History of CVD and Gender. The stratified analysis according to history of CVD showed that men with prior CVD had significantly better control of BP, weight, lipid profile, and smoking than men without a history of CVD (all variables $P < 0.001$) (Table 1). Additionally, there were no clinically significant differences with regard to glycemic control between the groups ($P = 0.058$). However, this pattern was strikingly different among women: those with previous CVD had significantly higher HbA1c (7.2% versus 7.1%; $P = 0.003$), systolic BP (mean 138 mmHg versus 137.5 mmHg; $P < 0.001$), and triglyceride values (156.3 mg/dL versus 153.1 mg/dL; $P < 0.001$) than women without a history of CVD.

When considering the adequate treatment goals of CVRFs by gender, women showed worse overall control than men ($P < 0.005$ for all variables except for smoking); this was seen both in subjects with no previous CVD and in those with history of any CVD ($P < 0.001$ for all studied variables) (Table 1 and Figure 1). The greatest differences compared with men were seen in the levels of LDL-cholesterol and in weight, while differences in BP were less evident, and the percentage of patients with HbA1c ≤ 7% was slightly higher among women without CVD (56.8% versus 56%, $P < 0.05$) and lower in women with CVD (54.6% versus 55%, $P < 0.05$). In accordance, the degree of composite control of CVRFs, that is, simultaneously taking into account hyperglycemia (HbA1C ≤ 7%, BP ≤ 140/90 mmHg) and LDL-cholesterol levels (LDL-cholesterol ≤ 130 mg/dL in patients without previous CVD and ≤100 mg/dL in those with prior CVD), was significantly worse among women: 25.1% of women without CVD were in good control compared to 27% of men, and among those with prior CVD, 17.7% of women had an optimal composite control versus 22.8% of men ($P < 0.005$ in both cases). Moreover, the proportion of patients with good composite control of CVRFs was lower among those with prior CVD, and this was true for both men and women: 17.7% of women with prior CVD were in good control versus 21.5% without CVD ($P < 0.001$), and 22.8% of men with prior CVD were in good control versus 27% without CV ($P < 0.001$).

3.2. Multivariate Analysis of Good CVRF Control according to Gender and CVD. After adjusting for gender (woman), age, diabetes duration, and treatment received, multivariate analysis showed that men in secondary prevention after CVD had better control of all risk parameters except for smoking. In the case of prevention of CVD, women still had better control over smoking than men, but also better control of their BP, whilst there were no clinically significant differences in glycemic control between genders (Table 2), and women remained worse than men at controlling weight and LDL-cholesterol levels.

3.3. Degree of CVRF Control in Different CVDs. Study of the different macrovascular complications, specifically coronary heart disease (CHD), stroke, or peripheral arterial disease (PAD), showed that the proportion of women with good control of target CVRFs, namely, HbA1c ≤ 7%, BP ≤ 140/90 mmHg, and BMI ≤ 30 Kg/m^2, and also lipid profiles in subjects with or without prior CVD was lower than men irrespective of the type of CVD ($P < 0.001$ in all cases) (Table 3).

3.4. Treatment of CVRFs in Patients with and without CVD according to Gender. We further studied whether treatment for the different CVRFs differed between genders in the presence/absence of prior CVD (Table 4). Among the subset of patients without a history of CVD, women had higher rates of prescribed glucose-lowering, antihypertensive, and lipid-lowering drugs than men (75% versus 73.3%, 70.8% versus 59.9%, and 47.6% versus 43.1%, resp.) and similar use of antiplatelet agents (27.6% versus 28.3%). However, in the subgroup of patients with a history of CVD, differences in the use or intensity of glucose-lowering and lipid-lowering treatments were not clinically relevant between genders, but women used less antiplatelet agents (71.8% versus 77.5%) and more antihypertensive agents (88.4% versus 86.4%) than men. Of note was that oral glucose-lowering agents in mono- or combined therapy were less often prescribed to women than to men in favor of a greater use of insulin therapy, either alone or combined with oral glucose-lowering drugs.

4. Discussion

Gender differences among the diabetic population include disparities in adherence to treatment [42], in control of cardiometabolic parameters and risk of CVD [30, 31, 43], and also in the therapeutic management of cardiovascular risk factors [25, 44, 45].

The prevalence rates of T2DM and CVD in our study were higher among men, which is in line with previous population-based studies [30, 46–48], although rates vary depending on the age range, country, and definition of CVD.

Table 1: Demographic, clinical characteristics, and degree of cardiometabolic control by gender and presence of CVD*.

	All patients		CVD		No CVD	
	Men $N = 153{,}987$	Women $N = 132{,}804$	Men $N = 34{,}283$	Women $N = 18{,}382$	Men $N = 119{,}704$	Women $N = 114{,}422$
CV risk factors						
Age, mean (SD), years	66.4 (11.3)	70.3 (11.1)	70.9 (9.6)	75.6 (8.7)	65.1 (11.4)	69.4 (11.2)
Diabetes duration, mean (SD), years	6.2 (4.8)	6.9 (5.3)	7.3 (5.5)	8.3 (6.4)	5.9 (4.5)	6.7 (5.1)
Hypertension, %	58.6	69.7	69.5	81.5	55.4	67.8
Systolic BP, mean (SD), mmHg	136.9 (13.6)	137.5 (14)	136.1 (14.3)	138 (14.7)	137.2 (13.4)	137.5 (13.8)
Diastolic BP, mean (SD), mmHg	76.6 (8.5)	76.2 (8.1)	73.8 (8.4)	73.6 (8.2)	77.5 (8.3)	76.6 (8)
Diabetic retinopathy, %	5.6	6.1	8.3	10.9	4.8	5.4
Diabetic nephropathy, %	20.7	12.3	26.7	18.3	19	11.3
BMI, mean (SD), kg/m^2	28.8 (4.3)	30.5 (5.6)	28.6 (4.1)	30.1 (5.4)	28.9 (4.3)	30.6 (5.6)
HbA1c, %	7.2 (1.5)	7.1 (1.4)	7.1 (1.4)	7.2 (1.4)	7.2 (1.5)	7.1 (1.4)
Total cholesterol, mean (SD), mg/dL	186.2 (38.2)	198.4 (38)	171.5 (36.9)	185.6 (39.4)	190.5 (37.5)	200.4 (37.3)
HDL-cholesterol, mean (SD), mg/dL	46.2 (12.3)	52.7 (13.4)	44.4 (11.8)	50.2 (12.9)	46.7 (12.4)	53.1 (13.4)
LDL-cholesterol, mean (SD), mg/dL	109.7 (32.2)	115.6 (32.3)	97.1 (30.7)	104.4 (32.5)	113.6 (31.6)	117.4 (31.9)
Triglycerides, mean (SD), mg/dL	158.5 (117.3)	153.5 (88.7)	153.4 (106.8)	156.3 (91.8)	160 (120.3)	153.1 (88.2)
Smoking status, %						
Non smokers	43.5	88	42.4	90.1	43.8	87.6
Current smokers	23.9	6.2	18.1	3.8	25.6	6.6
Ex-smoker	32.6	5.8	39.5	6.1	30.6	5.8
Degree of CVRF control						
HbA1c ≤ 7%, %	55.8	56.5	55	54.6	56	56.8
BMI ≤ 30 kg/m^2, %	61	47.3	62.5	49.7	60.6	46.9
BP ≤ 140/90 mmHg, %	63.9	63.1	65.5	62.1	63.5	63.2
LDL-cholesterol ≤ 130 mg/dL, % (PP)	75.2	69.4	86.3	80.2	71.8	67.7
LDL-cholesterol ≤ 100 mg/dL, % (SP)	41.3	34.2	58.8	49.2	35.9	31.7
HbA1c ≤ 7% + BP ≤ 140/90 mmHg + LDL ≤ 130 mg/dL, %	28.5	25.6	33.1	28.9	27.0	25.1
HbA1c ≤ 7% + BP ≤ 140/90 mmHg + LDL ≤ 100 mg/dL, %	15.7	12.4	22.8	17.7	13.5	11.5

*All variables showed significant differences between sexes ($P < 0.005$) and between CVD and no CVD in both men and women ($P < 0.001$), except for HbA1c: $P = 0.058$ in men and $P = 0.003$ in women. BMI: body mass index; BP: blood pressure; CVD: cardiovascular disease; PP: primary prevention; SD: standard deviation; SP: secondary prevention.

Table 2: Multivariate analysis on the degree of control of CVRFs stratified according to the presence of CVD.

	CVD		No CVD	
	OR[a] (95% CI)*	*P* value	OR[a] (95% CI)*	*P* value
HbA1c ≤ 7%	0.95 (0.91–1.00)	0.041	1.01 (0.99–1.03)	0.23
PA ≤ 140/90 mmHg	0.879 (0.84–0.92)	<0.001	1.082 (1.06–1.13)	<0.001
LDL-cholesterol ≤130 mg/dL (CVD) ≤100 mg/dL (no CVD)	0.67 (0.64–0.70)	<0.001	0.74 (0.72–0.76)	<0.001
BMI ≤ 30 Kg/m^2	0.50 (0.48–0.52)	<0.001	0.53 (0.52–0.54)	<0.001
Nonsmoker	4.20 (3.86–4.58)	<0.001	4.01 (3.39–4.13)	<0.001

BMI: body mass index; BP: blood pressure; CVD: cardiovascular disease; OR: odds ratio.
*OR[a]: odds ratio adjusted by age, diabetes duration, treatment received, and sex (women).

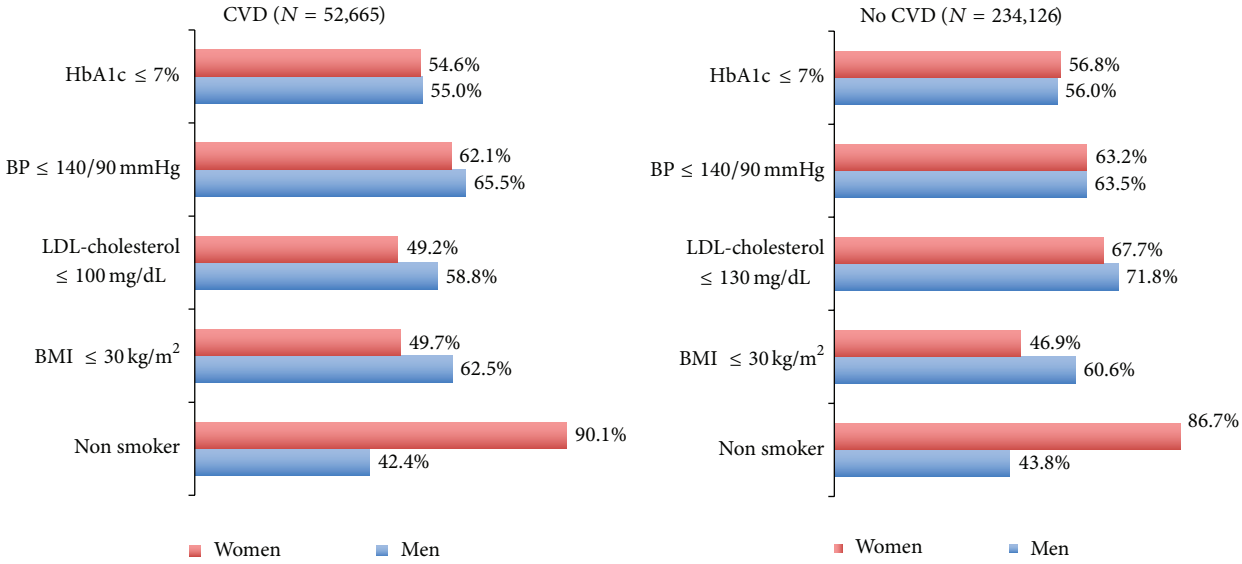

Figure 1: Percentage of patients with T2DM and good control of CVRF by gender and history of CVD (all variables showed significant differences between sexes ($P < 0.005$) and between CVD and no CVD in both men and women ($P < 0.001$), except for HbA1c: $P = 0.058$ in men and $P = 0.003$ in women. BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease).

The results of the study showed that there were significant gender differences in the control of T2DM and CVD individual risk factors. Namely, compared with men, women were on average older and had a longer duration of disease, and apart from less frequently being smokers than men, they had poorer control of hypertension, LDL-cholesterol levels, and BMI. This profile of worse control of CVRFs has been consistently reported before in previous surveys conducted in Spain and in other countries [30–32, 39, 43, 49–52], but the present study is the largest one ever performed in real-life clinical practice. Moreover, the proportion of women who achieved the target of stipulated recommendations to control the risk of CVD in our study was lower than men except for glycemic control, and the composite control of multiple risk outcomes (Hb1Ac, BP, and LDL-cholesterol simultaneously) was also poorer among women. These results are also in agreement with the above mentioned studies and with results from studies specifically assessing gender differences in composite risk factors in T2DM [43, 53], which have found that women are approximately 3 times less likely to achieve combined cardiometabolic control than men [43].

There are few reports assessing the control of CVRFs in T2DM according to gender as well as for the presence of prior CVD, and the present study is the first one conducted in a Spanish population. Our analysis stratifying by presence of prior CVD showed that both men and women with CVD in general had poorer control of CVRFs than those without. As for the degree of control of modifiable CVRFs, multivariate analysis showed that women with prior CVD were less likely to achieve their therapeutic targets than men for all parameters except for smoking. Women without CVD achieved the recommended HbA1c target as optimally as men and were better at controlling BP and smoking but again more frequently did not achieve recommended therapeutic targets for obesity and LDL-cholesterol. Our results on patients with prior CVD are in agreement with a previous cross-sectional study conducted in Germany, which found that women were more likely to have uncontrolled systolic BP,

Table 3: Degree of CVRFs control (% and 95% CI) in different macrovascular complications according to sex.

	CHD $N = 32{,}313$		Stroke $N = 18{,}768$		PAD $N = 8{,}420$		CHD + stroke $N = 3{,}670$		CHD + PAD $N = 2{,}312$		Stroke + PAD $N = 1{,}265$		CHD + stroke + PAD $N = 411$	
	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
HbA1c ≤ 7%	54.2 (53.4–54.9)	52.8 (51.7–53.9)	58.1 (57–59.1)	57.2 (56.0–58.5)	50.5 (49.1–51.9)	48.3 (45.8–50.8)	53.8 (51.5–56.0)	52.0 (48.8–55.2)	49.4 (46.8–52.0)	44.5 (39.0–50.1)	51.9 (48.5–55.4)	43.5 (36.5–50.6)	51.3 (45.3–57.4)	46.8 (32.0–61.6)
BP ≤ 140/90 mmHg	67.3 (66.0–66.7)	62.6 (61.7–63.6)	64.2 (63.3–65.2)	63.0 (61.9–64.2)	61.0 (59.8–62.3)	55.8 (53.5–58.1)	65.8 (63.8–67.8)	64.5 (61.1–67.4)	65.0 (62.8–67.3)	59.0 (53.9–64.0)	63.2 (60.0–66.3)	56.0 (49.5–62.5)	65.0 (59.7–70.4)	55.7 (42.9–68.6)
Nonsmoker	43.8 (43.1–44.4)	90.5 (89.9–91.0)	45.2 (44.2–46.2)	90.8 (90.1–91.4)	30.7 (29.6–31.9)	85.7 (84.1–87.3)	45.1 (43.1–47.2)	91.8 (90.2–93.4)	34.3 (32.1–36.5)	89.3 (86.2–92.5)	33.1 (30.1–36.1)	87.6 (83.3–91.8)	37.5 (32.2–42.9)	88.5 (80.3–96.8)
BMI ≤ 30 kg/m^2	39.7 (38.9–40.5)	52.4 (51.2–53.5)	34.3 (33.3–35.4)	47.8 (46.4–49.2)	33.0 (31.7–34.4)	47.7 (45.0–50.3)	34.0 (31.8–36.2)	50.8 (47.2–54.3)	35.9 (33.4–38.4)	47.3 (41.3–53.2)	31.9 (28.6–35.3)	44.0 (36.2–51.8)	31.1 (25.4–36.8)	53.5 (38.0–69.0)
LDL ≤ 130 mg/dL	89.1 (88.6–89.5)	82.8 (82.0–83.7)	84.9 (84.1–85.7)	78.9 (77.8–79.9)	82.2 (81.1–83.2)	73.7 (71.4–75.9)	90.9 (89.6–92.9)	84.4 (82.0–86.8)	89.8 (88.2–91.4)	81.5 (76.9–86.0)	86.1 (83.6–88.5)	79.0 (73.0–85.0)	88.0 (84.0–92.1)	81.0 (68.6–93.3)
LDL ≤ 100 mg/dL	63.1 (62.3–63.8)	53.2 (52.0–54.3)	56.4 (55.3–57.5)	46.8 (45.5–48.1)	51.8 (50.4–53.2)	42.2 (39.6–44.7)	65.1 (62.9–67.3)	54.7 (51.4–57.9)	62.4 (59.8–65.0)	58.4 (52.6–64.1)	58.4 (54.9–62.0)	48.6 (41.3–56.0)	59.8 (53.7–65.9)	54.8 (39.1–70.5)

BMI: body mass index; BP: blood pressure; CHD: coronary heart disease; PAD: peripheral artery disease.

Table 4: Treatment (%) used to control the different CVRFs in patients with or without CVD by gender.

Treatment	All patients $N = 286{,}791$			CVD $N = 52{,}665$			No CVD $N = 234{,}126$		
	Men $N = 153{,}987$	Women $N = 132{,}804$	P value	Men $N = 34{,}283$	Women $N = 18{,}382$	P value	Men $N = 119{,}704$	Women $N = 114{,}422$	P value
Glucose-lowering									
Lifestyle changes only	24.6	24.1	0.003	17.8	18.2	0.021	26.6	25.1	<0.001
Oral monotherapy	36.3	34.5	<0.001	33.8	29.2	<0.001	37.0	35.4	<0.001
Combination of OAD	22.9	21.9	<0.001	23.5	19.9	<0.001	22.7	22.2	0.001
Insulin + OAD	8.80	11.7	<0.001	13.3	18.1	<0.001	7.50	10.7	<0.001
Insulin monotherapy	7.37	7.80	<0.001	11.7	14.7	<0.001	6.10	6.70	<0.001
Any pharmacological treatment	*75.4*	*75.9*	*0.003*	*82.3*	*81.9*	*0.021*	*73.3*	*75*	*<0.001*
Antihypertensive									
No treatment	34.2	26.8	<0.001	13.6	11.6	<0.001	40.1	29.2	<0.001
ACE inhibitor/ARA2	16.3	14.9	<0.001	12.3	11.4	0.004	17.5	15.5	<0.001
Diuretic	2.01	4.4	<0.001	1.37	2.45	<0.001	2.20	4.72	<0.001
Beta-blocker	2.59	1.91	<0.001	5.60	3.37	<0.001	1.73	1.68	0.04
Calcium-channel blocker	2.19	2.15	0.43	3.82	3.28	0.001	1.73	1.97	<0.001
Combination of 2	22.7	26.8	<0.001	30.4	29.8	0.17	20.5	26.3	<0.001
Combination of 3 or more	19.5	22.8	<0.001	32.6	37.9	<0.001	15.7	20.3	<0.001
Any pharmacological treatment	*65.8*	*73.2*	*<0.001*	*86.4*	*88.4*	*<0.001*	*59.9*	*70.8*	*<0.001*
Lipid-lowering									
No treatment	50.3	49.4	<0.001	27.1	31.1	<0.001	56.9	52.4	<0.001
Statin	40.5	43.3	<0.001	60.2	58.9	0.003	34.8	40.8	<0.001
Fibrate	3.88	2.81	<0.001	2.36	2.17	0.17	4.31	2.91	<0.001
Statin + fibrate	1.06	0.67	<0.001	1.81	1.13	<0.001	0.84	0.59	<0.001
Any pharmacological treatment	*49.7*	*50.6*	*<0.001*	*72.9*	*68.9*	*<0.001*	*43.1*	*47.6*	*<0.001*
Antiplatelet									
No treatment	60.7	66.3	<0.001	22.5	28.2	<0.001	71.7	72.4	<0.001
Aspirin	31.4	28.8	<0.001	52.2	51.2	0.02	25.4	25.2	0.19
Clopidogrel	4.14	2.98	<0.001	12.6	11.9	0.01	1.71	1.55	0.004
Any pharmacological treatment	*39.3*	*33.7*	*<0.001*	*77.5*	*71.8*	*<0.001*	*28.3*	*27.6*	*<0.001*

ACE: acetylcholinesterase; ARA2: angiotensin II receptor antagonist; BMI: body mass index; BP: blood pressure; CVD: cardiovascular disease; OAD: oral antidiabetic drugs.

LDL-cholesterol, and HbA1c levels [25]; similarly, another cross-sectional analysis conducted in the US found that women were more liable to have suboptimal control of systolic BP and LDL-cholesterol but found no differences in glycemic control relative to men [45]. As for patients without prior CVD, the US study found no significant differences in the degree of control of any studied modifiable CVRF [45], and the German study only found a higher probability of women having uncontrolled LDL-cholesterol relative to men [25], which is in agreement with our results, although we also found that women had even better control of BP than men. Unfortunately, our results on smoking and BMI cannot be compared with these 2 studies, since both of them included these 2 risk factors as confounding covariates in their analysis.

There is compelling evidence in Spain and other countries that women receive less health care attention not only for the treatment of their T2DM [54], but also for the prevention and treatment of associated CV complications [14, 19, 25, 26, 30, 44, 45], as women both with and without CVD receive less lipid-lowering and antithrombotic therapy than men [29, 47, 55, 56]. Studies stratifying by gender and comorbid CVD are scarce but concur that women are less intensively treated with lipid-lowering drugs than men, in patients both with and without prior CVD, while findings on gender disparities according to prior CVD regarding the use or intensity of treatment with antihypertensive or glucose-lowering drugs are inconsistent across reports [25, 45, 47]. Differences between studies may be due to genetic or ethnic differences, geographical variations in access to available health care resources, different ambulatory physician practices between countries, and disparities in the economic barriers to care due to the type of insurance (public or private) paying for the treatment.

When we assessed whether there were gender disparities in the management of modifiable CVRF in T2DM patients according to a history of CVD, we found that women were more likely to be treated with antihypertensive drugs and less likely to take antiplatelet drugs than men irrespective of having a history of CVD, while glucose- and lipid-lowering treatment varied according to the absence/presence of prior CVD: the proportion of women with CVD taking glucose and/or lipid-lowering medications was similar to men, but women without CVD took more glucose and/or lipid-lowering drugs than men. However, while the degree of achieved glycemic control was similar between women with and without previous CVD, lipid levels were remarkably uncontrolled in both cases and more pronounced in women with prior CVD. This is of concern if we take into account that a history of CVD is an independent factor associated with higher morbidity and mortality and that the 4-year survival rate of women with prior CVD is lower than in women without a history of CVD [30]. Moreover, the fact that women without prior CVD did not achieve adequate control of lipid levels, in spite of being more likely to be treated with lipid-lowering medications than men, could be related to the use of less intensive therapy or to a differential response to statins relative to men, although this is controversial in the case of primary prevention [57, 58]. With regard to the degree of control of BP, we observed that women without CVD had similar control to men, in spite of higher levels of treatment with antihypertensive drugs, while women with CVD still had uncontrolled BP relative to men although they were treated in a comparable proportion, an observation that has been previously reported [29]. This is also of concern if we consider that women have a higher lifetime risk of stroke than men, in part because they have a longer life expectancy and because the risk of stroke increases with age [59], therefore, underlining the need for more intensive or proper control of BP. Taken together, our results show that the treatment and control of the 2 parameters that most effectively prevent CVD, namely, BP and lipid levels, remain a challenge (particularly LDL-cholesterol levels) in the case of women with T2DM and a history of CVD.

Strengths of the present study include the use of registries coming from primary care medical records, which allows the collection of a large volume of patients' real-life clinical practice data. However, there are some limitations that should be acknowledged and considered when interpreting the results of this study. Firstly, inherent to any cross-sectional design, no causal associations or conclusion on trends in treatment can be drawn, and the retrospective design is subject to biases concerning the lack of data recording for some of the studied variables (e.g., 25% of patients did not have corresponding HbA1c values for the previous year). Secondly, the studied cohort is representative of a specific territory in Spain and may not necessarily reflect standards of care in other territories. Thirdly, information on treated (and the specific therapeutic agents prescribed) and untreated patients was based on drugs obtained at the pharmacy, and we were not able to assess medication adherence factors. Finally, we had no data to assess factors known to differ by gender in T2DM that may influence disease outcomes, such as diabetes knowledge, self-management practices, lifestyle related factors, socioeconomic status, education, or social support [51].

5. Conclusions

The results of the study confirm that Spanish women with T2DM have suboptimal control of CVRFs; they also show that compared with men women with CVD were less likely to achieve therapeutic goals for BMI, BP, LDH-cholesterol, and HbA1c and that those without a history of CVD were also less likely to achieve BMI and LDL-cholesterol recommended goals. Furthermore, although the proportion of women treated with lipid-lowering medications was similar to or even higher than men, LDL-cholesterol levels were remarkably uncontrolled in both women with and without CVD, and women with CVD still had uncontrolled BP relative to men in spite of being treated with antihypertensive drugs in a comparable proportion of cases. The observed differences have clinical implications that warrant further investigation through studies specifically designed to assess gender differences in the control of modifiable CVRF and further stress the need to implement measures to better prevent and treat this subgroup of diabetic women. Actions should include not only targeted awareness programs for health professionals, but also the implementation of specific

educational programs aimed at improving self-awareness and self-care in women with T2DM.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Sexual Dysfunction in Women with Diabetic Kidney

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Few studies address alteration of sexual function in women with diabetes and chronic kidney disease (CKD). Quality of life surveys suggest that discussion of sexual function and other reproductive issues are of psychosocial assessment and that education on sexual function in the setting of chronic diseases such as diabetes and CKD is widely needed. Pharmacologic therapy with estrogen/progesterone and androgens along with glycemic control, correction of anemia, ensuring adequate dialysis delivery, and treatment of underlying depression are important. Changes in lifestyle such as smoking cessation, strength training, and aerobic exercises may decrease depression, enhance body image, and have positive impacts on sexuality. Many hormonal abnormalities which occur in women with diabetes and CKD who suffer from chronic anovulation and lack of progesterone secretion may be treated with oral progesterone at the end of each menstrual cycle to restore menstrual cycles. Hypoactive sexual desire disorder (HSDD) is the most common sexual problem reported by women with diabetes and CKD. Sexual function can be assessed in women, using the 9-item Female Sexual Function Index, questionnaire, or 19 items. It is important for nephrologists and physicians to incorporate assessment of sexual function into the routine evaluation protocols.

1. Introduction

The prevalence and incidence of diabetes is rather similar in the two sexes [1]. However, the long-term impact of diabetes on complication is more gender specific. Men in comparison with women seem to be at higher risk for microvascular complications, such as nephropathy [2], severe retinopathy [3], and sexual dysfunction [4]. Diabetic nephropathy is a major complication of diabetes mellitus (DM), affecting about 15%–25% of type 1 and 30%–40% of type 2 diabetic patients, and diabetic nephropathy accounts for approximately 44% of all cases of end stage renal disease (ESRD) [5]. Disturbances in sexual function are associated frequently with diabetic nephropathy and CKD [6]. Male patients with diabetic nephropathy and CKD suffer from reduced libido, erectile dysfunction, and difficulty reaching the orgasm. In females with diabetic kidney, dyspareunia, amenorrhea, reduction of libido, and a delay in sexual development are frequently observed. Sexual function of women with diabetes, however, has received less attention in clinical studies. Moreover results are less conclusive than studies in men, likely due to several factors, including a lack of standardized definitions of female sexual dysfunction, absence of well-validates scales, and social taboos regarding female sexuality [7].

2. Female Sexual Dysfunction

Female sexual dysfunction (FSD) has been described in diabetic women since the early 1980s. Sexual disorders reported in women with diabetes include the reduction or loss of sexual interest or desire, arousal or lubrication difficulties, dyspareunia, and loss of the ability to reach orgasm [7].

FSD is defined as a disorder of sexual desire, arousal, orgasm, and/or sexual a pain. In 2010, the Third International Consensus of Sexual Medicine accepted revised definitions of FSD, emphasizing a model based on a circular pattern

of the sexual female response, in which different phases of sexual function can overlap. More recently, the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5), released newer and revised definitions, in which sexual desire and arousal disorders have been combined into the "female sexual interest/arousal disorder" category, and vaginismus and dyspareunia have been grouped into the "genitopelvic pain/penetration disorder" category. Moreover, all of the sexual dysfunctions outlined in the DSM-5 require a minimum duration of approximately 6 months, and more precise severity criteria must be met in order to provide useful thresholds for making a diagnosis and for distinguishing transient sexual difficulties from more persistent sexual dysfunction [8, 9].

FSD has been associated with both type 1 and type 2 diabetes. A recent meta-analysis that included 26 studies, 3,168 diabetic women, and 2,823 controls showed that FSD is more frequent and is associated with a lower Female Sexual Function Index (FSFI) score in diabetic women than in controls. In particular, the risk for FSD was 2.27 (95% confidence interval (CI): 1.23–4.16) and 2.49 (95% CI: 1.55–3.99) in type 1 and type 2 diabetic women, respectively. Furthermore, the risk for FSD was 2.02 (CI: 1.49–2.72) when considering "any diabetes" (which represented the two forms of diabetes together). Interestingly, an increased risk of FSD was found in premenopausal women with "any diabetes," but not in postmenopausal women. Moreover, at the statistical analysis among the independent variables, only BMI was significantly associated with the FSFI effect size ($P = 0.005$), suggesting that the higher frequency of FSD and lower FSFI score found in diabetic women may be related to body weight. Several studies have already shown an increased prevalence of FSD in women affected by obesity and metabolic syndrome [10]. Studies that have focused on type 1 diabetic women provided a valid opportunity to investigate the role of diabetes on sexual function, independent of other associated comorbidities. In type 1 diabetic women, FSD appears to be correlated mainly psychological factors, such as depression, anxiety, and marital status [11]. Results from a large prospective study of 625 women with type 1 diabetes showed that depression was the major predictor of sexual dysfunction [11].

3. Pathophysiology

Pathogenesis of FSD is related to decreased libido, low arousability, decreased vaginal lubrification, orgasmic dysfunction, and dyspareunia. The causes implicated in such disturbances are peripheral neurological disease, vascular impairment, and psychological complaints.

Several studies showed that circulating concentration of estradiol are decreased in women with type 1 DM, suggesting that diabetes may be associated with dysregulation of sex hormone biosynthesis [12]. Similar observations were made in patients with CKD and type 1 DM. Women with diabetes type 1 have impaired ovarian function, delayed age at menarche, more risk of menstrual irregularities, and complication in pregnancy such as spontaneous abortion, stillbirths, and congenital anomalies, when age matched with nondiabetic women. A set of validated instruments including the Female Sexual Distress Scale (FSDS) and Beck's Inventory for Depression (BDI) were used as basic methods. During the follicular phase, patients and control subjects had similar FSFI scores. During the luteal phase, patients had significantly lower FSFI scores and significantly higher FSDS scores, while BDI was equal. During the follicular phase, patients had lower estrogenic profile, as well as delta4-androstenedione, DHEAS, and fT4 and fT3 than control subjects. During the luteal phase, total testosterone levels were higher in patients than control subjects, while 17-Beta estradiol and progesterone levels were lower in patients than in control subjects [13].

The endocrinological basis of FSD in diabetic disease was mostly investigated in the study conducted by Salonia et al. [13]. Sexual function and endocrine profile in women with type 1 diabetes were studied during follicular and luteal phases of menstrual cycle and compared to control group.

In women with diabetic nephropathy and CKD, decrease of libido, amenorrhea, disturbances in menstruation, and fertility are caused by elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Midcycle LH surge cannot be mitigated with endogenous administration of estrogen, confirming a central hypothalamic derangement. The major menstrual abnormality in diabetes and CKD is anovulation, with consequence of infertility. Clinical manifestations of sexual dysfunction in women include premature menopause, skin wrinkling, urinary incontinence, hot flushes, sleep and cognitive disorders, and cardiovascular disease [12]. The reduction of libido is frequently observed, while the pregnancy is rare (spontaneous abortion is a common eventuality). Few studies carefully examined ovarian function in women with diabetic nephropathy; this lack of data reflects probably the complexity of studying the reproductive system in women. Both the smooth muscle relaxation of female genitalia erectile tissue and the enhancement of genital blood flow are dependent upon the action of nonadrenergic/noncholinergic neurotransmitters, such as vasoactive intestinal polypeptide b (VIP) and nitric oxide (NO). The regulation of blood flow and clitoral erectile function is governed by the same NO/cyclic guanosine monophosphate (cGMP) pathway in women as well as that involved in erectile function in men. NO and phosphodiesterase type 5 (PDE5) have been identified in human clitoral smooth muscle, indicating a key role of NO in female sexual function [14]. Normal levels of various hormones are also required for physiologic sexual activity. Diabetes and CKD may affect all of these integrated systems, leading to sexual dysfunction. The mechanisms involved include hyperglycemia, infections, vascular and neurological damage, and hormonal disorders [15].

4. Sex Hormones in the Diabetic kidney

Several studies focused on the association between sex hormones and diabetic renal disease. First, puberty is the turning point for the development of diabetic nephropathy. Second, the protection of the female sex against the development of renal disease is missing in setting of diabetes [16]. Third, CKD, secondary to diabetes, is associated with sexual dysfunction

and altered sex hormone profiles. Despite the fact that it is unclear whether diabetic nephropathy is characterized by an imbalance in sex hormone levels and whether restoring this imbalance may be renoprotective, few studies tested the effects of estrogens in diabetic nephropathy [6, 17]. Short-term administration of estrogens in recombination with a synthetic progestin has been shown to reduce proteinuria and improved creatinine clearance in postmenopausal women with type 2 diabetes. Similarly, the Insulin Resistance Atherosclerosis Estradiol exerts its actions through both Er alfa and Er beta receptors, which are predominants in the kidney of female rats, whereas only Er beta receptors are predominants in male [16]. Not all studies, however, support the beneficial effects of estrogens in the diabetic kidney.

The use of oral contraceptives, containing high doses of estrogen, has been linked to the development of macroalbuminuria [16].

Ovarian failure in women with diabetic kidney and CKD can be associated with abnormalities at several sites in the hypothalamic-pituitary-ovarian axis.

During the follicular phase of the ovarian cycle, baseline plasma levels of estradiol, progesterone estradiol, and FSH are comparable between premenopausal women with diabetic disease. During the midcycle LH surge, however, the LH levels of premenopausal women with diabetes and CKD are far below those of normal women. After administration of clomifene, which can be used to evaluate the responsiveness of the hypothalamic-pituitary axis, plasma levels of LH and FSH increase significantly in patients, which indicates that the negative-feedback effect of estrogen on the hypothalamus is intact. Secretion of gonadotropin-releasing hormone (GNRH) has both tonic and cyclic components. The tonic component regulates basal gonadotropin secretion and is controlled by negative estradiol feedback mechanism. The cyclic component is dependent on estrogen secretion; the increase in estrogen levels in the middle of the menstrual cycle is responsible for enhanced secretion of GNRH and subsequent LH surge [6, 17].

After an estrogen stimulation test, normal individuals experience a surge in plasma LH levels; by contrast, plasma LH levels do not rise and plasma FSH levels are suppressed after administration of estrogen to women with diabetic nephropathy. The absence of an increase in LH levels in these patients strongly indicates a defect in the positive hypothalamic feedback mechanism.

High levels of serum prolactin, present in 80% of women with diabetes and CKD, might contribute to ovulatory dysfunction and decreased libido in this population.

5. Psychological Factors

Psychological factors can have a substantial effect on the sexual function in patients with diabetes and CKD. It has been noted in several studies that 20–30% of patients with diabetes and CKD suffer from clinical depression. Psychometric profile includes mood deflection, major depression, interpersonal issue and psychological aspect of living with diabetes and CKD [17].

Two major studies by Enzlin et al. [11] showed that women with DM1 either with or without SD reported more depressive symptoms than men with and without SD, respectively.

Enzlin et al. [11] reported a significantly higher incidence of depressive symptoms in women with DM1 who had sexual problem than in women without sexual dysfunction [11]. However, depression is a well-known determinant of decreased desire impaired arousability in women with diabetes. Salonia et al. suggest, according to the Female Sexual Distress Scale (a 12-item instrument to measure sexually related personal distress in women, that sexual distress is similar between patients and control subjects during the follicular phase. In women with DM1 sexual distress correlated significantly with a reduction in libido scores ($r = -0.44$, $P = 0.034$) and lower lubrification scores ($r = 0.45$, $P = 0.032$).

Associations were found between FSD and a variety of other quality of life parameters, such as mental and physical components of the 36-item short-form (SF-36) health survey and depression scores.

Depression is strongly associated with diabetes. Most epidemiological studies showed that psychosocial factors are the main contributors to sexual dysfunctions in both type 1 and type 2 diabetes. Depression seems to be the main determinant of sexual dysfunction in women with diabetes. Diabetic complications may also affect health and relationship status, quality of life, and a woman's self-image, generating a vicious cycle that may have detrimental effects on sexual performance.

6. Diagnosis and Evaluation of Sexual Dysfunction

The first step in the evaluation of sexual dysfunction in patients with diabetes and CKD is to obtain a detailed sexual history about the sexual desire, arousal and orgasmic capabilities, and fertility. Changes in the frequency of intercourse need to be determined. Often the patients are very reluctant to tell such concerns. The physicians should determine the time of the onset of these problems in relation to the stage of disease [17–19]. The domains of sexual function in women include desire, arousal, pain, and satisfaction. These can be assessed using the 9-item FSFI. There are several validated screening tools that focus on HSDD, which is the most common sexual concern of women of all ages (Table 1) [20–22].

In addition, the medical history should focus on the patient's past and present medical illness, that is, chronic/medical illness, such as anemia; neurological illness or lumbosacral disc disease; endocrinological disease, like hypogonadism, hyperprolactinemia, and thyroid disorders; atherosclerotic vascular risk, hypercholesterolemia, hypertension, hyperhomocysteinemia, smoking habits, or family history. Current drug therapy should also be reviewed in detail. Drugs such as cimetidine, tricyclic antidepressant, phenothiazines, and metoclopramide are often implicated in SD in female.

Finally, it is important to investigate patients for presence of psychosocial problems (depression, psychiatric illness) and current stressors factor (loss of job or home and so on).

Table 1: Screening tools for FSD.

(i) Decreased Sexual Desire Screener (DSDS): 5 questions, self-administered, assesses for generalized acquired HSDD [29].
(ii) Female Sexual Function Index (FSFI): 19 questions, self-administered, assesses all of the dimensions of female sexual function including sexual satisfaction [20].
(iii) Sexual Interest and Desire Inventory-Female (SIDI-F): 13 items, clinician administered, assesses severity of female HSDD [21].
(iv) Brief Hypoactive Sexual Desire Disorder Screener: 4 questions, self-administered HSDD in postmenopausal women [21].
(v) Brief Profile of Female Sexual Function (B-PFSF): 7 questions, self-administered HSDD in postmenopausal women [21].
(vi) Female Sexual Distress Scale-Revised (FSDS-R): 13 questions, self-administered, assesses distress associated with female SD [19, 21, 22, 26, 30].

Table 2: What the guidelines say you should do: treatment of sexual dysfunction in women and the opportunity for psychosexual and/or couples counseling.

(i) The generalized use of testosterone by women has been advised against, because of inadequate indications and lack of long-term data. However, postmenopausal women who are distressed by their decreased sexual desire and who have other identifiable causes may be candidates for testosterone therapy. Androgens which may also be used by those women are hypogonadal as a result of pituitary problems in premenopause.
(ii) Although there is no consistent correlation between sexual functioning and levels of androgens (free and total testosterone, androstenedione, dehydroepiandrosterone, and SHBG) across wide age range, in some women androgen therapy can improve sexual desire.
(iii) Transdermal patches and topical gel or creams are preferred over oral products because of first pass hepatic effects documented with oral formulation.
(iv) The major side effects of androgens are hirsutism and acne. No safety with regard to testosterone implants. There is no indication for increased frequency of breast cancer [20, 21, 27–29].

7. Management of Sexual Dysfunction in Women

Few studies address decreased libido and sexual function in women with diabetic nephropathy and CKD. Surveys on "quality of life" suggest that discussion of sexual function and other reproductive issues are key components of psychosocial assessment and that education on sexual function in the setting of diabetic nephropathy and CKD is widely needed [23]. Pharmacologic therapy with estrogen/progesterone and androgens along with correction of anemia, ensuring adequate dialysis delivery, and treatment of underlying depression are important. Changes in lifestyle such as smoking cessation, strength training, and aerobic exercises may decrease depression, enhance body image, and have positive impacts on sexuality [24, 25]. Women with diabetic nephropathy who suffer from chronic anovulation and lack of progesterone secretion may be treated with oral progesterone at the end of each menstrual cycle to restore menstrual cycles. It is not clear whether unopposed estrogen stimulation of the endometrium (due to anovulatory cycles) predisposes women with diabetic nephropathy to endometrial hyperplasia or endometrial cancer. Routine gynecologic follow-up is recommended in these cases, and some women may also benefit from the use of a progestational agent several times a year to mitigate the effects of estrogen on the endometrium [23–28].

Low estradiol levels in amenorrhoeic women on diabetes leads to vaginal atrophy and dyspareunia. Topical estrogen cream and vaginal lubricants may be helpful in this situation. Women with diabetic nephropathy who do have menstrual cycles should be encouraged to use contraception; because of poor pregnancy outcomes, restoring fertility is not an advisable therapeutic goal. HSDD is the most common sexual problem reported by women with diabetes and CKD. Testosterone replacement therapy to treat HSDD has been effective in some women without diabetes and CKD [29]. However, long-term safety data on the use of androgens in women with diabetes and CKD are very limited (Table 2).

8. Conclusions

FSD pathogenesis in diabetes is complex, and current studies have not yet clarified all of the pathological pathways involved; these studies are limited by the small sample sizes, lack of standardized definitions of sexual dysfunction, and inadequate characterization of diabetes, especially regarding glycemic control, the presence of complications, and the presence of depression. In contrast to what is described in men, female sexual function appears to be more related to social and psychological components than to the physiological consequence of diabetes. In conclusion, psychological concerns may play a significant role in the development of FSD in both type 1 and type 2 diabetes. This is in line

with the complex nature of female sexuality, which is largely dependent on psychological and cultural factors, even more so than male sexuality.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Markers of Systemic Inflammation and Apo-AI Containing HDL Subpopulations in Women with and without Diabetes

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Background. Besides their role in reverse cholesterol transport, HDL particles may affect the atherosclerotic process through the modulation of subclinical inflammation. HDL particles differ in size, composition, and, probably, anti-inflammatory properties. This hypothesis has never been explored in diabetic women, frequently having dysfunctional HDL. The potential relationship between lipid profile, Apo-AI containing HDL subclasses distribution, and common inflammatory markers (hsCRP, IL-6) was examined in 160 coronary heart disease- (CHD-) free women with and without type 2 diabetes. *Results*. Compared to controls, diabetic women showed lower levels of the atheroprotective large α-1, α-2, and pre-α-1 and higher concentration of the small, lipid-poor α-3 HDL particles ($P < 0.05$ all); diabetic women also had higher hsCRP and IL-6 serum levels (age- and BMI-adjusted $P < 0.001$). Overall, HDL subclasses significantly correlated with inflammatory markers: hsCRP inversely correlated with α-1 ($P = 0.01$) and pre-α-1 ($P = 0.003$); IL-6 inversely correlated with α-1 ($P = 0.003$), α-2 ($P = 0.004$), and pre-α-1 ($P = 0.002$) and positively with α-3 HDL ($P = 0.03$). Similar correlations were confirmed at univariate regression analysis. *Conclusions*. More atheroprotective HDL subclasses are associated with lower levels of inflammatory markers, especially in diabetic women. These data suggest that different HDL subclasses may influence CHD risk also through the modulation of inflammation.

1. Introduction

Cardiovascular disease is the primary cause of death also in diabetic women [1–4].

Low plasma levels of high-density lipoprotein cholesterol (HDL-C) have been largely recognized as a risk factor for coronary heart disease (CHD) [5, 6] and they are a common feature of insulin resistance states [7].

HDL class comprises very heterogeneous particles that can be separated by different methods, including two-dimensional gel electrophoresis that separates Apo-AI containing HDL particles according to their size and lipid content [5]. Specific particles have been shown to differently promote cholesterol efflux, suggesting a distinct role in reverse cholesterol transport (RCT) and CVD risk protection [5, 8–10]. Thus, Cheung et al. reported that the presence of CHD was more strongly associated with HDL particle size distribution than with low HDL-C level [11]. Furthermore, it was documented that low levels of α-1 and α-2 HDL particles were better predictors of CHD risk than total HDL-C concentration in both the Framingham Offspring Study and the VA-HIT study [12, 13].

We have recently shown that type 2 diabetes determines a shift in the distribution of HDL particles; in particular, when assessing HDL subpopulation distribution by two-dimensional gel electrophoresis, diabetic women had HDL that are selectively depleted in the large lipid-rich α-1, α-2, and pre-α-1 and enriched in the small, lipid-poor α-3 HDL subpopulations, resulting in HDL particles that were smaller in size and poor in cholesterol compared with those

of unaffected subjects; this profile resembled that of men with CHD participating in the Framingham Offspring Study [14].

Besides their role in RCT, HDL particles exert their antiatherosclerotic role through several other mechanisms, such as a reduction of inflammation, endothelial dysfunction, and LDL oxidation [15]. Thus, proteins involved in the inflammatory response, such as serum amyloid A (SAA), have been located in specific HDL subspecies [16–18].

Several inflammatory markers and adipokines have been subjected to intensive studies for their role in insulin resistance and atherosclerosis. In particular, high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) have been clearly involved in both insulin resistance and atherosclerosis prediction [19–24]. Despite the growing body of evidence indicating that determination of HDL subpopulations may add important information on CHD risk [5, 10], data on the potential role of different HDL subpopulations in the inflammatory process are still limited [25]. This information may be particularly valuable in type 2 diabetic women, whose HDL particles are typically dysfunctional, as we have recently demonstrated [14].

In that same population of CHD-free women with and without type 2 diabetes [14], we now further investigate the potential relationships between different HDL LpA-I and LpA-I:A-II subclasses and markers of systemic inflammation.

2. Methods

2.1. Study Subjects. Study population has been previously described elsewhere [14]. Briefly, eighty type 2 diabetic and 80 nondiabetic women were consecutively recruited among those attending the metabolic disease outpatient clinic of Messina University Hospital and from voluntary employees of the same institution. The two groups were matched for age (age range: 40–62 years) and menopause (37 pre- and 43 postmenopausal in each group).

Exclusion criteria for all participants were as follows: pregnancy, hormonal replacement therapy, oral contraceptive use or multivitamin supplementation, current treatment with β-blockers, fibrates, statins, omega 3 fatty acids, niacin, or anti-inflammatory drugs, fasting serum creatinine >1.5 mg/dL (>132.7 μmol/L), macroalbuminuria (Albustix positive), any major medical condition in the last 6 months preceding the study, and documented cardiovascular disease (CVD, defined as myocardial infarction, ischemic heart disease, coronary heart bypass, coronary angioplasty, cerebral thromboembolism, and peripheral amputations).

Lifestyle and clinical data were collected through a standardized questionnaire.

BMI and blood pressure (BP) were measured according to standard procedures. Type 2 diabetes was diagnosed according to ADA criteria [26]. Diabetic women had a mean duration of disease of 5.7 ± 6.7 years and a mean HbA_{1c} of 7.4 ± 1.5%. Subjects participating in the study were on the following diabetes therapies at enrolment: 4 (5.0%) were taking sulfonylureas alone, 24 (30.0%) were taking metformin alone, 32 (40.0%) were taking a combination of metformin and sulfonylureas, 8 (10.0%) were taking repaglinide, 2 (2.5%) were on insulin in combination with metformin and sulfonylureas, and 10 (12.5%) were not taking any medication for diabetes. None of the participants was on acarbose, glitazones, and/or incretins at the time of the study. Retinopathy was diagnosed in 15% of diabetic participants and 5% of them had microalbuminuria.

All the participants gave their informed consent and the study was approvedby the local ethical committee.

2.2. Biochemical Analyses. After a 12- to 14-hour fasting, blood samples were collected from all participants for the determination of the study parameters. Blood was drown in a 10 mL tube containing EDTA (0.15% final concentration) and in a regular 10 mL tube. After collection, plasma and serum were immediately separated at 2,500 rpm for 30 minutes at 4°C, and aliquots were stored at −80°C until analysis. Fasting plasma glucose and serum creatinine levels were measured with standard automated laboratory methods (Roche Diagnostics, Milan, Italy). Glycated haemoglobin (HbA_{1c}) was measured using an automated high-performance liquid chromatography (HPLC) analyzer (Diamat: Bio-Rad Laboratories, Milan, Italy); normal range values in our laboratory are 4–6%. Fasting insulin concentration was measured by radioimmunoassay (Diagnostic Corporation, LA, CA, USA).

2.3. Plasma Lipids, Lipoprotein, and HDL Subpopulation Measurements. All lipid and lipoprotein measurements were performed at the Lipid Metabolism Laboratory, Tufts University. Plasma total cholesterol (TC) and triglycerides levels were measured by automated enzymatic assays [27]. Direct low-density lipoprotein cholesterol (LDL-C) was measured with reagents from Equal Diagnostics (Exton, PA). HDL cholesterol (HDL-C) was measured directly with a kit from Roche Diagnostics (Indianapolis, IN). Very-low-density lipoprotein cholesterol (VLDL-C) was calculated with the following equation: VLDL-C – TC – (LDL-C + HDL-C).

Apo-A-I containing HDL subpopulations in plasma were measured by nondenaturing two-dimensional gel electrophoresis, as previously described [5]. Briefly, HDL were first separated by charge, on agarose gel, into pre-β, α, and pre-α-mobility particles. In the second dimension, each of these 3 fractions of HDL was further separated according to size (into preβ1 and 2, α1, 2, and 3, and preα-1, 2, and 3) by nondenaturing polyacrylamide gel electrophoresis. This was followed by transfer into a nitrocellulose membrane and immunoblotting with a monospecific anti-Apo-A-I primary antibody and a ^{125}I-labelled secondary antibody. Signals were quantitated by image analysis using a FluoroImager (Molecular Dynamics, Sunnyvale, CA). Apo-A-I concentrations of the subpopulations were calculated by multiplying the percent of each subpopulation by the plasma total Apo-A-I concentration. The CV was <10% for α particles and was <15% for all other subpopulations.

2.4. Markers of Systemic Inflammation. All inflammatory markers measurements were performed at the Lipid Metabolism Laboratory, Tufts University. Measurements of hsCRP were performed on a Hitachi 911 (Roche Diagnostics, Indianapolis, Indiana) using the hsCRP kit from Wako Chemicals. Within- and between-run coefficients of variation were

<5%. Plasma concentrations of interleukin- (IL-) 6 were determined by an ELISA assay (R&D Systems, Minneapolis, Minnesota).

2.5. Statistical Analysis. The numerical data are expressed as mean and standard deviation (SD). Examined variables were normally distributed as verified by Kolmogorov-Smirnov test; consequently, the parametric approach has been used. For each parameter, we performed statistical comparisons between women with and without diabetes applying Student's *t*-test. The Pearson correlation test was applied in order to assess the existence of significant interdependence between hsCRP and all numerical parameters, as well as IL-6 and all numerical parameters.

Finally, linear regression models were estimated in order to assess the possible dependence of hsCRP on all examined variables; firstly, we estimated all univariate models; subsequently, a multivariate regression analysis was performed including in the model only the variables significantly associated with inflammatory markers levels in the univariate approach. The same analysis was performed in order to assess the dependence of IL-6.

$P < 0.05$ was considered to be statistically significant.

Statistical analysis was performed using the SPSS program, version 11.0, for Windows (SPSS Inc., Chicago, IL).

3. Results

3.1. Lipid Profile, Apo-AI Containing HDL Subpopulations Distribution, and Markers of Systemic Inflammation in Women with and without Type 2 Diabetes. Clinical characteristics of the 160 CHD-free women, 80 with and 80 without type 2 diabetes, participating in the study have been previously described [14] and are shown in Table 1. Women participating in the study were matched for age, menopausal status, and menopause duration. Overall, type 2 diabetic women had higher BMI and waist circumferences, systolic and diastolic BP, and fasting plasma glucose than nondiabetic ones ($P < 0.001$ for all comparisons). These differences remained statistically significant after adjustment for age and BMI (Table 1).

As shown in Table 1, plasma concentration of triglycerides was higher ($P = 0.001$; age- and BMI-adjusted $P < 0.05$) and levels of HDL-C ($P < 0.0001$, also after age- and BMI-adjustment), Apo-AI ($P = 0.04$; not significant after adjustment for age and BMI), and Apo-AII ($P = 0.01$; age- and BMI-adjusted $P < 0.05$) were lower in diabetic women than in nondiabetic women.

When comparing circulating levels of principal Apo-AI containing HDL subpopulations (Table 1), α-1 ($P = 0.006$; age- and BMI-adjusted $P < 0.05$), α-2 ($P = 0.005$; age- and BMI-adjusted $P < 0.05$), and pre-α-1 HDL ($P = 0.02$; age- and BMI-adjusted $P < 0.05$) were significantly lower and α-3 HDL ($P = 0.02$; age- and BMI-adjusted $P < 0.05$) levels were significantly higher in diabetic women than in control women.

Diabetic women also had 2-fold higher hsCRP serum levels than nondiabetic ones (age- and BMI-adjusted $P < 0.001$); similarly, also IL-6 serum levels (age- and BMI-adjusted $P < 0.001$) were higher in diabetic women than in control women.

3.2. Correlations of Serum Levels of Markers of Systemic Inflammation with Metabolic Parameters, Lipid Profile, and Apo-AI Containing HDL Subpopulations Distribution in Women with and without Type 2 Diabetes. Overall, markers of systemic inflammation significantly correlated with metabolic and lipid parameters and HDL subpopulations. In particular, hsCRP levels positively correlated with IL-6 in both the diabetic group ($r = 0.50$; $P < 0.001$) and the control group ($r = 0.55$; $P < 0.001$).

As shown in Table 2, circulating hsCRP and IL-6 significantly correlated with BMI, waist circumference, fasting blood glucose, and insulin levels ($P < 0.05$ for all); IL-6 correlated with age and systolic and diastolic BP.

In the whole study population, both hsCRP and IL-6 positively correlated with triglycerides and inversely correlated with HDL-C and Apo-AII concentrations; IL-6 also showed inverse correlation with Apo-AI levels ($P < 0.05$ for all).

Significant correlations of inflammatory markers with specific Apo-AI containing HDL subclasses were also noted. Notably, hsCRP and IL-6 showed significant inverse correlations with the larger lipid-rich α-1, α-2, and pre-α-1 HDL subclasses and a positivecorrelation with the smaller, lipid-poor α-3 HDL particles. In particular, hsCRP inversely correlated with α-1 ($P = 0.01$) and pre-α-1 ($P = 0.003$); IL-6 negatively correlated with α-1 ($P = 0.003$), α-2 ($P = 0.004$), and pre-α-1 ($P = 0.002$) and positively with α-3 ($P = 0.03$).

Similar correlations were also noted when separately considering diabetic women and controls, although these correlations were less numerous, especially in controls (Table 2).

In particular, in women with diabetes, hsCRP significantly correlated with BMI ($P < 0.001$), waist circumference ($P < 0.001$), and fasting insulin ($P < 0.001$) and negatively correlated with creatinine ($P = 0.025$); moreover, hsCRP levels showed a significant correlation also with triglycerides ($P = 0.012$) and an inverse correlation with HDL-C ($P = 0.018$) and Apo-AII ($P = 0.037$) and with α-1 ($P < 0.05$) and pre-α-1 HDL subclasses ($P < 0.05$). IL-6 levels significantly correlated with BMI ($P = 0.002$), systolic ($P = 0.036$) and diastolic BP ($P = 0.018$), and fasting insulin ($P < 0.001$) and negatively correlated with Apo-AII ($P = 0.016$) and α-2 HDL subclasses ($P = 0.018$).

In women without diabetes, hsCRP levels showed a significant correlation with BMI ($P < 0.001$), waist circumference ($P < 0.001$), fasting blood glucose ($P = 0.029$), and fasting insulin ($P < 0.001$), whereas no significant correlation was noted with lipid profile or HDL subfractions. IL-6 levels significantly correlated with age ($P = 0.035$), waist circumference ($P = 0.018$), systolic BP ($P = 0.038$), and fasting insulin ($P < 0.001$) and inversely correlated with Apo-AII ($P = 0.025$) and α-1 HDL subclasses ($P = 0.034$); the correlation of BMI with IL-6 was more significant in controls than in diabetic women ($P < 0.001$).

3.3. Univariate and Multivariate Regression Analysis between hsPCR and IL-6 Levels and Metabolic, Lipid, and Apo-AI HDL Particles Profile in Total Study Population. At univariate regression analysis (Table 3), diabetes, BMI, waist circumference, fasting blood glucose, and insulin levels were the factors significantly associated with hsCRP concentrations

Table 1: Lipid profile, Apo-AI containing HDL subpopulations distribution, and markers of systemic inflammation in women with and without type 2 diabetes.

	Total population	Women with type 2 diabetes	Women without type 2 diabetes	P
n	160	80	80	
Postmenopausal (n)	86	43	43	
Age (yrs)	51.32 ± 10.13	52.03 ± 9.70	50.61 ± 10.56	—
Menopausal duration (yrs)	9.04 ± 7.62	8.59 ± 6.67	9.35 ± 8.27	—
BMI (Kg/m^2)	29.52 ± 6.85	32.38 ± 6.91	26.47 ± 5.34	<0.001
Waist circumference (cm)§	95.46 ± 14.28	99.71 ± 12.58	88.67 ± 14.34	<0.001
Systolic BP (mmHg)*	126.79 ± 16.33	131.5 ± 18.01	121.84 ± 12.69	<0.001
Diastolic BP (mmHg)§	75.58 ± 9.16	78.75 ± 8.88	72.24 ± 8.26	<0.001
Fasting BG (mg/dL)*	129.02 ± 46.23	160.54 ± 47.16	97.11 ± 5.22	<0.001
Lipid and lipoprotein profile				
Total-C (mg/dL)$^{\#}$	191.81 ± 29.32	190.86 ± 29.44	192.75 ± 29.36	—
LDL-C (mg/dL)$^{\#}$	124.97 ± 27.82	124.46 ± 27.23	125.5 ± 28.60	—
Triglycerides (mg/dL)§	104.50 ± 63.37	120.7 ± 78.2	88.2 ± 37	0.001
HDL-C (m/dL)*	51.78 ± 13.40	47.35 ± 13.58	56.22 ± 11.71	<0.0001
Apo-AI (mg/dL)$^{\#}$	125.94 ± 19.78	122.75 ± 20.69	129.14 ± 18.34	0.04
Apo-AII (mg/dL)§	31.09 ± 4.82	30.14 ± 5.24	32.04 ± 4.18	0.01
Apo-AI containing HDL subpopulations profile				
α-1 HDL (mg/dL)§	21.33 ± 9.47	19.32 ± 8.97	23.35 ± 9.58	0.006
α-2 HDL (mg/dL)§	43.38 ± 9.52	41.29 ± 9.62	45.47 ± 8.99	0.005
α-3 HDL (mg/dL)§	17.27 ± 4.81	18.18 ± 5.56	16.36 ± 3.74	0.02
Pre-α-1 (mg/dL)§	6.13 ± 3.47	5.51 ± 3.39	6.74 ± 3.46	0.025
Markers of systemic inflammation				
hsCRP (mg/L)*	4.31 ± 6.34	5.93 ± 7.66	2.68 ± 4.11	0.001
IL-6 (pg/mL)*	2.30 ± 2.69	2.70 ± 3.23	1.9 ± 1.94	—

Data are n, means ± SD. Only significant P values for the comparisons between diabetic and nondiabetic women are presented. Total-C: total cholesterol; Apo: apolipoprotein. *Age- and BMI-adjusted P value < 0.001; §age- and BMI-adjusted P value < 0.05; $^{\#}$nonsignificant age- and BMI-adjusted P value.

in the whole study population. Circulating hsCRP was also inversely associated with HDL-C and Apo-AII concentrations and with α-1 and pre-α-1 HDL particles.

IL-6 was significantly associated with BMI, waist circumference, systolic BP, fasting blood glucose, and insulin levels and negatively with HDL-C, Apo-AI, and Apo-AII levels. A trend was also noted for an association with diabetes. IL-6 also showed significant associations with almost all the HDL subclasses explored, specifically a negative association with α-2 and pre-α-1 particles and a positive association with α-3 HDL subfractions.

Multivariate regression analysis was performed including in the model only HDL subpopulations and not HDL-C levels, to avoid colinearity. As a result (Table 3), BMI was the only factor significantly associated with hsCRP concentrations in the whole study population. However, a trend was noted for an inverse association of hsPCR levels with pre-α-1 HDL particles. BMI and fasting plasma glucose were significantly associated with IL-6 levels, whereas no significant association was described with lipid profile.

4. Discussion

Low levels of HDL-C are a mainstay of diabetic dyslipidemia and a largely recognized CHD risk factor [28–30], especially in insulin resistant patients [31].

HDL particles may be particularly atheroprotective in women, where each 1 mg/dL increase in HDL-C is associated with a 3% decrease in CHD risk versus 2% in men [6].

The antiatherosclerotic role of these particles may be also mediated by the modulation of inflammation, since atherosclerosis today is considered an inflammatory disease.

We have recently shown that diabetic women have a less atheroprotective HDL subpopulation pattern [14]. In this study, we investigated the potential relationship of HDL-C levels, HDL subclasses, and hsPCR and IL-6 levels, two well-known markers of inflammation, in that cohort of CHD-free women with and without type 2 diabetes [14].

Both hsCRP and IL-6 are well-characterized inflammatory markers in type 2 diabetes [19–24], being independently related to insulin resistance [32] and to the progression of atherosclerosis [33]. Women usually have higher hsCRP levels than men [34, 35], probably as a consequence of their relatively higher degree of visceral adiposity. In our female study population, both hsCRP and IL-6 levels were higher in diabetic women than in controls, although this difference was statistically significant only for hsCRP. These observations are largely consistent with previous studies showing a high degree of subclinical inflammation in the presence of diabetes [25, 36]. Furthermore, gender differences were also reported in these associations. Thus, in the Mexico City Diabetes Study [37], hsCRP levels were associated with incident diabetes in

Table 2: Correlation coefficients (r_S) between markers of systemic inflammation and metabolic, lipid, and Apo-AI containing HDL subpopulations profile in women with and without type 2 diabetes.

	Total population		Women with type 2 diabetes		Women without type 2 diabetes	
	hsCRP	IL-6	hsCRP	IL-6	hsCRP	IL-6
Age	—	0.17*	—	—	—	0.24*
Menopause duration	—	—	—	—	—	—
BMI	0.62§	0.48§	0.55§	0.35*	0.58§	0.49§
Waist C	0.57§	0.37§	0.45§	—	0.53§	0.35*
Systolic BP	—	0.30§	—	0.24*	—	0.24*
Diastolic BP	—	0.29§	—	0.27*	—	—
Fasting BG	0.35§	0.35§	—	—	0.25*	—
Fasting insulin	0.51§	0.43§	0.45§	0.36§	0.52§	0.44§
Creatinine	—	—	−0.25*	—	—	—
Lipid and Apo-AI containing HDL subpopulations profile						
Total-C	—	—	—	—	—	—
LDL-C	—	—	—	—	—	—
Triglycerides	0.28§	0.19*	0.28*	—	—	—
HDL-C	−0.23*	−0.29§	−0.26*	—	—	—
Apo-AI	—	−0.19*	—	—	—	—
Apo-AII	−0.19*	−0.32§	−0.23*	−027*	—	−0.25*
α-1 HDL	−0.19*	−0.24*	−0.22*	—	—	−0.24*
α-2 HDL	—	−0.33*	—	−0.27*	—	—
α-3 HDL	—	0.18*	—	—	—	—
Pre-α-1 HDL	−0.023*	−0.24*	−0.21*	—	—	—

Only significant correlation coefficients (Spearman test) are shown. §P, P value < 0.001; *P, P value < 0.05. Waist C: waist circumference; BP: blood pressure; BG: blood glucose, Total-C: total cholesterol; Apo: apolipoprotein.

women but not in men, and in the MONICA/KORA study the association of hsCRP with the risk of type 2 diabetes was stronger in women [38].

Our results also confirm the association of inflammatory markers with adiposity and insulin resistance, since BMI, waist circumference, fasting blood glucose and insulin, and systolic and diastolic BP were all significantly associated with inflammatory markers, especially in women with diabetes. However, these associations are probably driven by the deleterious effects of obesity, since at multivariate analysis BMI was the strongest correlate of inflammatory markers in our dataset, even more than diabetes itself, which was no longer significant at multivariate analysis, although fasting blood glucose was still significantly associated with IL-6 levels.

When the potential relationships between inflammation and lipid profile, with particular regard to HDL particles, were assessed, we found significant correlations between inflammatory markers and HDL-C levels and Apo-AI and Apo-AII concentrations, especially in diabetic women, whereas no associations were noted with other lipid fractions. Although many of these correlations disappeared when separating women with and without diabetes, probably because of the smaller sample size, these results are in accordance with those of the ATTICA study, where a significant correlation between HDL-C concentrations and markers of systemic inflammation was shown [25]. Accordingly, familial low-HDL-C subjects display higher levels of hsCRP [39].

The potential anti-inflammatory role of HDL particles is sustained by several lines of evidence. Thus, besides their role in RCT, HDL particles may have several other antiatherosclerotic mechanisms, including the modulation of oxidation, inflammation, and endothelial dysfunction [40].

Indeed, as reported in numerous studies [41, 42], HDL may stimulate nitric oxide and prostacyclin production from endothelial cells, regulate vascular structure and tone, promote endothelial survival [43], and influence immunity, modulating the expression of inflammatory chemokines and complement system [44]. Furthermore, the numerous enzymes carried by these lipoproteins, such as paraoxonase, platelet-activating factor-acetyl hydrolase, LCAT, or glutathione seleno peroxidase, prevent LDL oxidation and confer HDL anti-infectious properties [44, 45].

The anti-inflammatory properties of HDL particles have been also sustained by proteomics analysis, revealing more than 50 proteins associated with HDL, most of which are with specific anti-inflammatory or antioxidant functions.

However, the link between inflammation and HDL particles is complex. Thus, the protective role of HDL-C levels appears to be attenuated by acute or chronic inflammation [46].

In vitro studies have shown that HDL isolated from coronary artery disease (CAD) subjects are able to exert proinflammatory properties when compared to particles isolated from controls [47].

Table 3: Univariate and multivariate regression analysis between hsCRP and IL-6 and metabolic, lipid, and Apo-AI containing HDL subpopulations profile in total population.

	hsPCR				IL-6			
	Univariate regression		Multivariate regression		Univariate regression		Multivariate regression	
	B	*P*	*B*	*P*	*B*	*P*	*B*	*P*
Anthropometric and metabolic parameters								
Diabetes	3.251	0.001	—	—	0.800	0.061	—	—
BMI	0.41	<0.001	0.24	0.03	0.14	<0.001	0.141	0.003
Waist C	0.11	0.001	—	—	0.03	0.02	—	—
Systolic BP	—	—	—	—	0.03	0.01	—	—
Fasting BG	0.03	0.005	—	—	0.01	0.009	0.011	0.02
Fasting insulin	0.09	0.004	—	—	0.04	0.009	—	—
Lipid and Apo-AI containing HDL subpopulations profile								
HDL-C	−0.10	0.005	—		−0.05	0.002	—	
Apo-AI	—	—	—	—	−0.03	0.003	—	—
Apo-AII	−0.21	0.04	—	—	−0.13	0.004	—	—
α-1 HDL	−0.11	0.04	—	—	—	—	—	—
α-2 HDL	—	—	—	—	−0.06	0.009	—	—
α-3 HDL	—	—	—	—	0.11	0.04	—	—
Pre-α-1 HDL	−0.39	0.007	−0.34	0.083	−0.13	0.03	—	—

Only significant *P* are presented. Waist C: waist circumference; BP: blood pressure; BG: blood glucose; Apo: apolipoprotein.

Anti-inflammatory effects of HDL particles may be also particularly relevant in acute coronary syndrome (ACS), where vascular inflammation strongly affects plaque vulnerability [48]. Thus, a significant shift in the HDL proteome of ACS subjects was observed, with modifications in several proteins including Apo-AIV, C3 complement, HDL-associated haemoglobin, and SAA [49]. Thus, the apoprotein and enzyme constituents of HDL can be replaced by acute phase reactants (serum amyloid A, fibrinogen), which attenuates the capacity of HDL to mediate other antiatherogenic functions [49]. However, whether the "inflammatory" state of HDL is able to impair their ability in RCT is still a matter of debate [49, 50].

These and other experimental lines of evidence indicate that, under inflammatory conditions, HDL particles lose their protective capacity shifting toward a proatherogenic pattern [51, 52], probably because of HDL remodelling, leading to modifications in composition and structure of HDL particles [51, 53, 54]. All these lines of evidence suggest the necessity of determining the "quality" of HDL particles more than estimating their quantity [5, 55], a concept that has led some authors to define an "inflammatory index" to quantify the pro- or anti-inflammatory profile of HDL [56, 57].

It is becoming apparent that different HDL particles may show peculiar "qualities" that may influence RCT process, as well as their antioxidant or anti-inflammatory potential, rendering them atheroprotective or proatherogenic [58].

In CHD patients, Asztalos et al. showed distinct alterations in HDL subpopulation distribution, as assessed by nondenaturing two-dimensional electrophoresis [5]. Accordingly, in our group of CHD-free type 2 diabetes women, we previously observed these same alterations in HDL subpopulation distribution, with a reduction of large lipid-rich α-1, α-2, and pre-α-1 HDL and an increase of the small, lipid-poor α-3 HDL subpopulations [14].

Since these modifications could negatively influence anti-inflammatory properties of HDL particles, we also tested the hypothesis that different HDL LpA-I and LpA-I:A-II subclasses may be differently associated with inflammation. Our data confirm this hypothesis, since markers of inflammation negatively correlated with large lipid-rich α-1 and α-2 HDL subfractions, which are considered more atheroprotective. IL-6 levels also positively correlated with the small α-3 HDL concentrations, which show proatherogenic properties. These correlations were more evident in women with diabetes. Notably, low levels of α-1 HDL particles have been shown to be the most significant predictor of recurrence of cardiovascular events in CHD patients [59], and the negative association of this HDL subfraction with hsCRP levels observed in our study suggests that the modulation of inflammation may play a crucial role.

Although the small sample size is a limitation, in our study population, the use of lipid-lowering medications, anti-inflammatory drugs, and glitazones was accurately excluded to avoid their confounding effect on the relationship between inflammatory markers and lipid variables.

Another limitation is the cross-sectional design of our study that does not allow us to determine whether a specific HDL profile is less "anti-inflammatory" or, on the contrary, it is the higher inflammatory state which modifies HDL particles distribution toward a proatherogenic pattern.

In conclusion, our data show that HDL-C and the more atheroprotective HDL subpopulations are inversely associated with inflammatory markers, suggesting that different HDL particles may exert a different role in inflammation.

However, caution must be taken when interpreting these associations that need to be confirmed in larger populations.

The functionality of HDL particles is a matter of growing investigation and, while waiting for validated markers in the clinical practice, the measurement of specific HDL subfractions might be useful to better evaluate the CVD risk in diabetic subjects.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Effects of a New Flavonoid and Myo-Inositol Supplement on Some Biomarkers of Cardiovascular Risk in Postmenopausal Women: A Randomized Trial

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Background and Aim. Cardiovascular risk is increased in women with menopause and metabolic syndrome. Aim of this study was to test the effect of a new supplement formula, combining cocoa polyphenols, myo-inositol, and soy isoflavones, on some biomarkers of cardiovascular risk in postmenopausal women with metabolic syndrome. *Methods and Results*. A total of 60 women were enrolled and randomly assigned ($n = 30$ per group) to receive the supplement (NRT: 30 mg of cocoa polyphenols, 80 mg of soy isoflavones, and 2 gr of myo-inositol), or placebo for 6 months. The study protocol included three visits (baseline, 6, and 12 months) for the evaluation of glucose, triglycerides, and HDL-cholesterol (HDL-C), adiponectin, visfatin, resistin, and bone-specific alkaline phosphatase (bone-ALP). At 6 months, a significant difference between NRT and placebo was found for glucose (96 ± 7 versus 108 ± 10 mg/dL), triglycerides (145 ± 14 versus 165 ± 18 mg/dL), visfatin (2.8 ± 0.8 versus 3.7 ± 1.1 ng/mL), resistin (27 ± 7 versus 32 ± 8 μg/L), and b-ALP (19 ± 7 versus 15 ± 5 μg/mL). No difference in HDL-C concentrations nor in adiponectin levels between groups was reported at 6 months. *Conclusions.* The supplement used in this study improves most of the biomarkers linked to metabolic syndrome. This Trial is registered with NCT01400724.

1. Introduction

Cardiovascular disease (CVD) events remain the leading cause of mortality and are a major cause of morbidity and disability in both genders worldwide [1]. CVDs cover a collection of various heart and vascular diseases, among these hypertension, coronary heart, and cerebrovascular diseases represent major public health problems. It is well known that in postmenopausal CVD risk is increased, since the protective effects exerted by estrogens are missing. It is a matter of fact that following menopause, negative changes in blood pressure, glucose level, and lipid profile are frequent, and characterize the so called "metabolic syndrome", responsible for increased CVD risk [2]. Furthermore, the majority of postmenopausal women experience an undesirable weight gain. CVDs have multiple causes; however, most of the events originate from the complications of atherosclerosis, a pathophysiological process that can be prevented by dietary habit [3]. Evidence from epidemiological studies indicates a positive association between reduction in the incidence of CVD and consumption of plant-based foods such as fruit and vegetables [4]. In addition, the relationship between the amount of polyphenol-rich food consumption (fruit and fruit juices, tea, wine, and cocoa) and chronic diseases, supports a protective effect of these compounds upon CVD [5]. The meta-analysis by Desch and colleagues [6] has confirmed the blood pressure-lowering capacity of flavonol-rich cocoa products, in a large set of trials. It has been estimated that a 3 mmHg reduction in systolic blood pressure (SBP) would reduce the relative risk of stroke mortality by 8%, coronary artery disease (CAD) mortality by 5%, and all-cause of mortality by 4% [7]. Other effects of cocoa-derived products include the improved platelet

function [8] and the reduction of LDL-cholesterol [4], the major atherogenic lipoprotein.

Besides cocoa flavanols, many other dietary substances have a proven efficacy in reducing CVD risk markers; in previous studies we already shown the protective effects of two naturally occurring molecules, the isoflavone genistein [9] and myo-inositol [10, 11], produced by the human body from glucose. Inositol is a polyol which may be considered a second messenger of insulin [12], and myo-inositol is one of its nine isomers, capable of reducing insulin resistance, blood pressure, and improving lipid profile in a small cohort of postmenopausal women affected by metabolic syndrome [10, 11]. The soy-derived isoflavone genistein acting as a natural selective estrogen receptor modulator (SERM) has a proven efficacy on markers of CVD risk [9] and in reducing bone loss in postmenopausal women [13]. Very recently, genistein has shown to reduce insulin resistance, blood pressure, and homocysteine and it improved lipid profile in a cohort of women with metabolic syndrome [14].

In light of these previous experiences and observations, the aim of this study was to test a new supplement formula, combining cocoa polyphenols, myo-inositol, and soy isoflavones, in postmenopausal women with metabolic syndrome. The rationale for this combination therapy is to offer a natural replacement therapy (NRT) that might improve the metabolic conditions in postmenopausal women. In particular, cocoa polyphenols are antioxidants; inositol is an insulin sensitizing agent and soy isoflavones have a positive effect also on bone metabolism.

2. Subjects and Methods

2.1. Enrollment and Randomization to Treatments. A 12-month, randomized, open-label study was performed. The study protocol was consistent with the principles of the Declaration of Helsinki and has been approved by the Ethics Committee of the University of Messina, Italy. All participants gave written informed consent. Women ($n = 60$) were recruited at the Menopause Outpatients of the University of Messina. Following the criteria for inclusion, women had to be 50 to 60 years old, postmenopausal for at least 12 months at baseline, and diagnosed with metabolic syndrome. Based on the NCEP ATP III [15], the diagnosis of metabolic syndrome in women requires three or more of the five following criteria: (1) waist circumference $\geq$ 88 cm; (2) triglycerides $\geq$ 150 mg/dL or on drug treatment for elevated triglycerides; (3) HDL-C $<$ 50 mg/dL or on drug treatment for reduced HDL-C; (4) fasting glucose $\geq$ 100 mg/dL or on drug treatment for hyperglycemia; (5) blood pressure $\geq$ 130/85 mmHg or on antihypertensive drug. At enrollment a complete family history was obtained and physical examination and routine laboratory evaluation were performed. Almost all the women were hypertensive (55/60) and in treatment with antihypertensive agents.

Exclusion criteria were clinical or laboratory evidence of confounding systemic diseases (e.g., chronic renal or hepatic failure and chronic inflammatory diseases); breast affections or familiar history of breast disease; CVD defined as documented myocardial infarction, ischemic heart disease, coronary heart bypass, coronary angioplasty, cerebral thromboembolism, peripheral amputations, and coagulopathy; use of oral or transdermal estrogen, progestin, androgens, selective estrogen receptor modulators, or other steroids; treatment in the preceding six months with polyunsaturated n-3 fatty acids supplements and nonsteroidal anti-inflammatory drugs (NSAIDs); smoking habit of more than 2 cigarettes daily. Furthermore, subjects were advised to report on occasional use of NSAIDs to the study investigators. Additional exclusion criteria included history of alcohol or drug abuse, being enrolled in another clinical study, proven hypersensitivity to the study drugs, and concomitant major diseases. A computer generated randomization with sequence random permuted blocks was used to minimize differences between groups, due to the limited number of women. Subjects were assigned to the intervention group (NRT: $n = 30$) or to the placebo group ($n = 30$).

All participants were counseled on a Mediterranean-style diet composed of 25% to 30% energy from fat, less than 10% from saturated fatty acids, 55% to 60% from carbohydrates, and 15% from protein, with a cholesterol intake less than 300 mg/d and fiber intake of 35 g/d or greater. We used this diet to avoid interference with the lipid profile. Diet adherence was evaluated in all participants during each follow-up visit. Soy products or other dietary supplements were prohibited whereas legumes' intake was suggested to be kept constant overtime. At least 150 minutes per week of moderately intense physical activity (walking or cycling) was recommended.

The treatment (herein after NRT) was given for 6 months and it was composed of 30 mg of cocoa polyphenols, 80 mg of soy isoflavones, and 2 grams of myo-inositol. Both NRT and placebo were in powder. In the following 6-month period only diet was recommended to both groups. Women were followed monthly with telephone calls to reinforce the adherence to the intervention.

The study protocol included three visits (baseline, 6, and 12 months) for evaluation of serum glucose, triglycerides, and HDL-C together with some adipokines: adiponectin, visfatin, and resistin; and a marker of bone turnover: bone-ALP.

2.2. Determination of the Study Variables. Primary outcome was the improvement (as a 20% change from baseline) of at least one of the parameters that characterize metabolic syndrome: blood pressure, waist circumference, HDL-cholesterol, triglycerides, and fasting glucose. Secondary outcome was the improvement of the studied markers as adiponectin, resistin, visfatin, and bone-ALP.

Fasting glucose was measured using routine colorimetric method (normal range 65–110 mg/dL). HDL-C and triglycerides were measured by using a routine enzymatic method (SGM Italia, Italy).

Adiponectin, resistin (all from DRG International Inc., Marburg, Germany), bone-specific alkaline phosphatase (IDS, ltd, Fountain Hills, AZ), and visfatin (Ucsn Life Science, Inc., Houston, TX) were measured using enzyme-linked immunosorbent assay kits from serum samples. Adiponectin

Table 1: Demographic and clinical characteristics of the study groups (mean ± SD).

	NRT $n = 30$ (basal)	Placebo $n = 30$ (basal)	P	NRT $n = 26$ (6 months)	Placebo $n = 24$ (6 months)	P
Age (years)	56.3 ± 3.8	55.5 ± 4.8	0.3			
Menopause (months)	95.1 ± 61.6	75.6 ± 57	0.2			
BMI (Kg/m^2)	31.9 ± 3.8	33.6 ± 3.9	0.1	30.7 ± 3.9	33.3 ± 3.3	0.2
Systolic blood pressure (mmHg)	123.6 ± 12	132.6 ± 20	0.09	124 ± 9.1	121.6 ± 9.8	0.3
Diastolic blood pressure (mmHg)	80 ± 8	81.5 ± 11.9	0.3	73.6 ± 11.2	70 ± 6.3	0.17
Waist circumference (cm)	98.6 ± 6.2	100.8 ± 12.6	0.2	98.8 ± 6	96.3 ± 6.9	0.17

Table 2: Biomarkers evaluation through the study (mean ± SD).

		Glucose (mg/dL)	HDL-C (mg/dL)	Triglycerides (mg/dL)	B-ALP (μg/mL)	Adiponectin (μg/mL)	Visfatin (ng/mL)	Resistin (μg/L)
NRT	Basal	110 ± 10	44 ± 7	177 ± 19	15 ± 6	18 ± 6	3.8 ± 1	35 ± 10
	6 months	96 ± 7*	50 ± 6	145 ± 14*	19 ± 7**	17 ± 4	2.8 ± 0.8***	27 ± 7**
	12 months	98 ± 6$^{\#\#}$	50 ± 5	156 ± 15§	26 ± 6$^{\#\#}$	19 ± 5$^{\#\#}$	2.9 ± 1$^{\#\#}$	26 ± 8§§
Basal versus 6 months	P value	<0.001	0.06	<0.001	<0.001	0.48	0.002	0.0016
Placebo	Basal	105 ± 7	45 ± 6	180 ± 20	16 ± 6	22 ± 5	4 ± 1	30 ± 9
	6 months	108 ± 10	46 ± 8	165 ± 18	15 ± 5	16 ± 7	3.7 ± 1.1	32 ± 8
	12 months	110 ± 8	48 ± 7	176 ± 22	12 ± 5	12 ± 6	4 ± 0.6	35 ± 10
Basal versus 6 months	P value	0.23	0.62	0.008	0.53	0.0013	0.32	0.42

$^{*}P < 0.001$ versus placebo 6 months; $P = 0.004$ versus placebo 6 and 12 months; $^{**}P = 0.02$ versus placebo 6 months; $^{***}P = 0.001$ versus placebo 6 months; $^{\#\#}P < 0.001$ versus placebo 12 months; $^{\S}P = 0.004$ versus placebo 12 months; $^{\S\S}P = 0.009$ versus placebo 12 months.

limit of detection was 0.2 μg/mL with an intra-assay CV of 7.5% and an interassay CV of 6.5%. Resistin limit of detection was 0.012 ng/mL with an intra-assay CV of 5.2% and an interassay CV of 7%. Visfatin limit of detection was 6.3 pg/mL with an intra-assay CV of 9% and an interassay CV of 10%. Bone-ALP limit of detection was 0.7 μg/L with an intra-assay CV of 4.5% and an interassay CV of 5.8%.

2.3. Adverse Events. Participants were also followed and monitored by their general practitioners (who served as external monitors), who received a detailed synopsis of the trial and were unaware of the treatment arm. At clinical visit every 6 months, women were asked about symptoms. Standard clinical evaluations and routinary laboratory analyses were done every 6 months. All unfavorable and unintended clinical effects were considered adverse events and were evaluated for severity, duration, seriousness, and relation to the study drug and outcome.

2.4. Statistical Analysis. The analysis-of-variance (ANOVA) for repeated measures was performed to analyze treatment effects on groups and time and the unpaired t-test was used to compare mean differences in continuous variables intra- and intergroups. A P value of 0.05 or less was considered statistically significant. A post hoc power calculation analysis demonstrated over 95% power when considering as primary endpoint either glycemia or triglycerides, with an alpha error of 0.05. Statistical analysis was performed by using Statistical Package for Social Science (SPSS Statistics 17.0 Chicago, IL) software.

3. Results

At the beginning of the study, the 2 groups were comparable for age, BMI, months from menopause, waist circumferences, and blood pressure (Table 1); and for clinical characteristics: serum glucose, HDL-cholesterol, and triglycerides (Table 2).

At the end of the 6 months treatment period, only 26 in the NRT group and 24 in the placebo group remained in the study (Figure 1). In the following six-month period other dropouts occurred, thus only 22 in the treated group and 21 in the placebo group completed the study. Reasons for withdrawal were abdominal pain (3 cases) and throat dryness (2 cases) and other reasons were not reported.

At 6 months, a significant difference between NRT and placebo was found for glucose, triglycerides, b-ALP, visfatin, and resistin as reported in Table 2 and Figures 2 and 3. No difference in HDL-C concentrations nor in adiponectin levels between groups was reported at 6 months (Table 2 and Figures 2 and 3). At 12 months, after 6 months from the end of the treatment period, in addition to glucose, triglycerides,

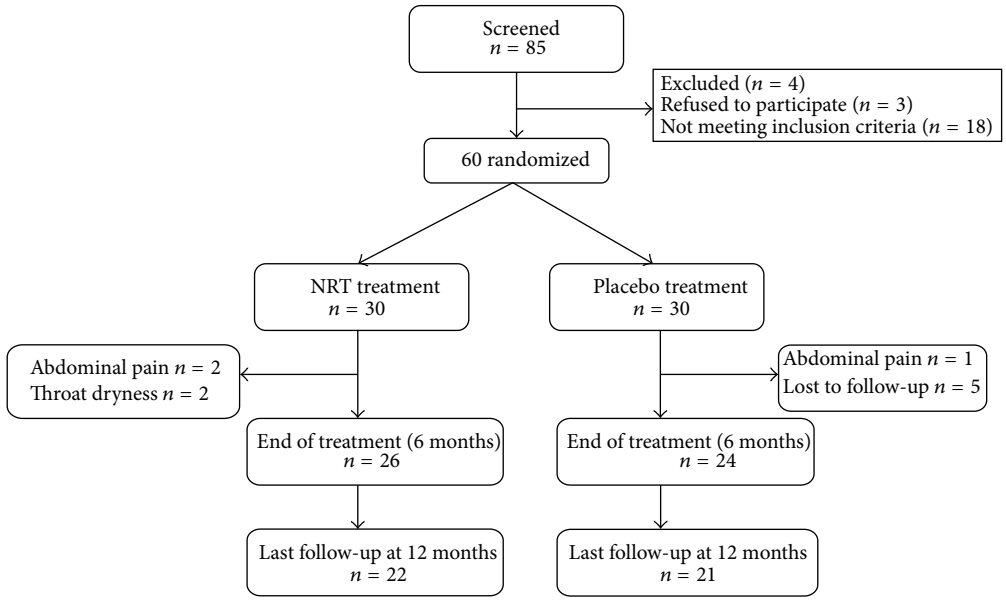

Figure 1: Study flow chart.

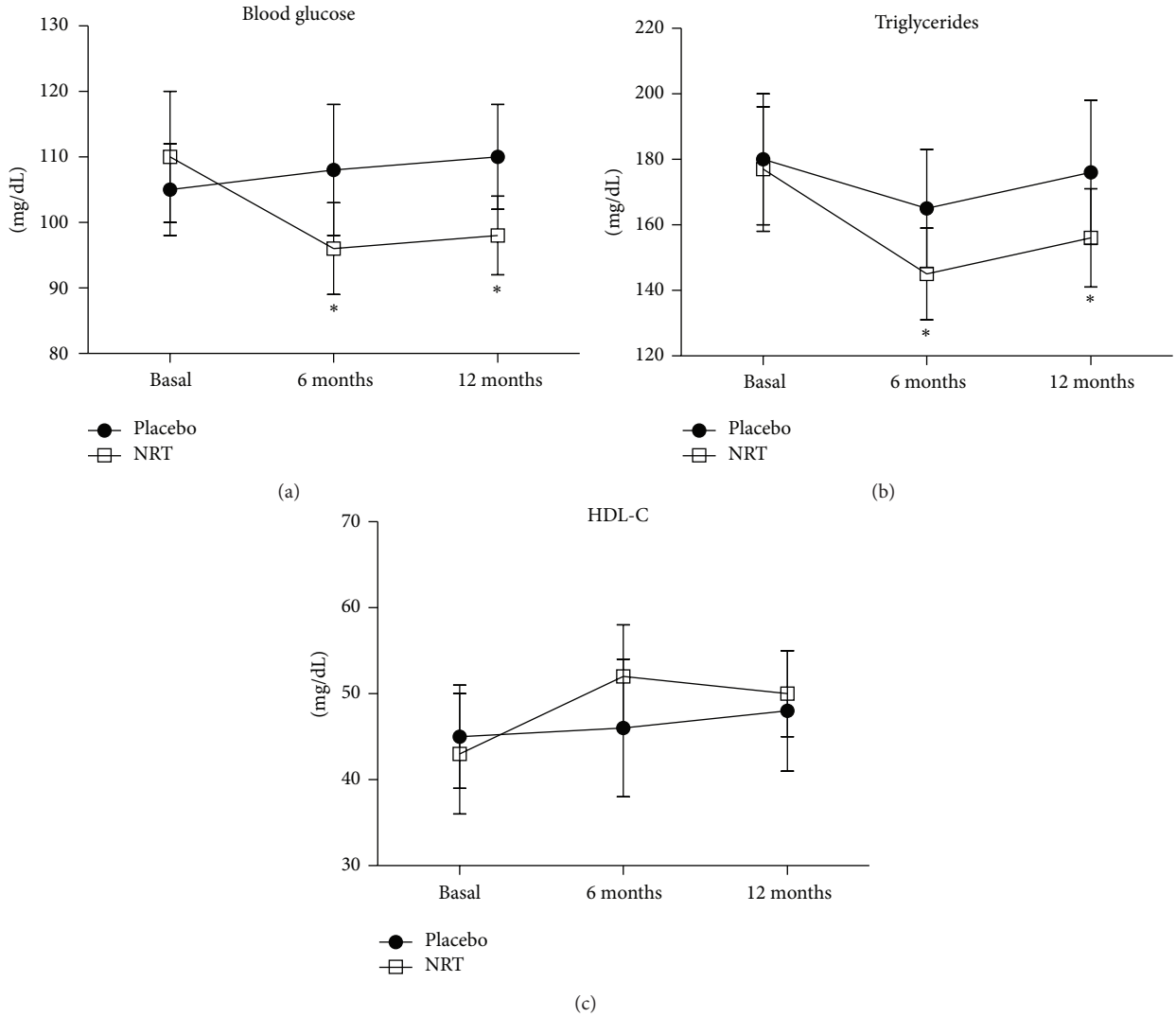

Figure 2: Glucose, triglycerides and HDL-cholesterol blood levels through the study. $^{*}P < 0.05$ versus basal.

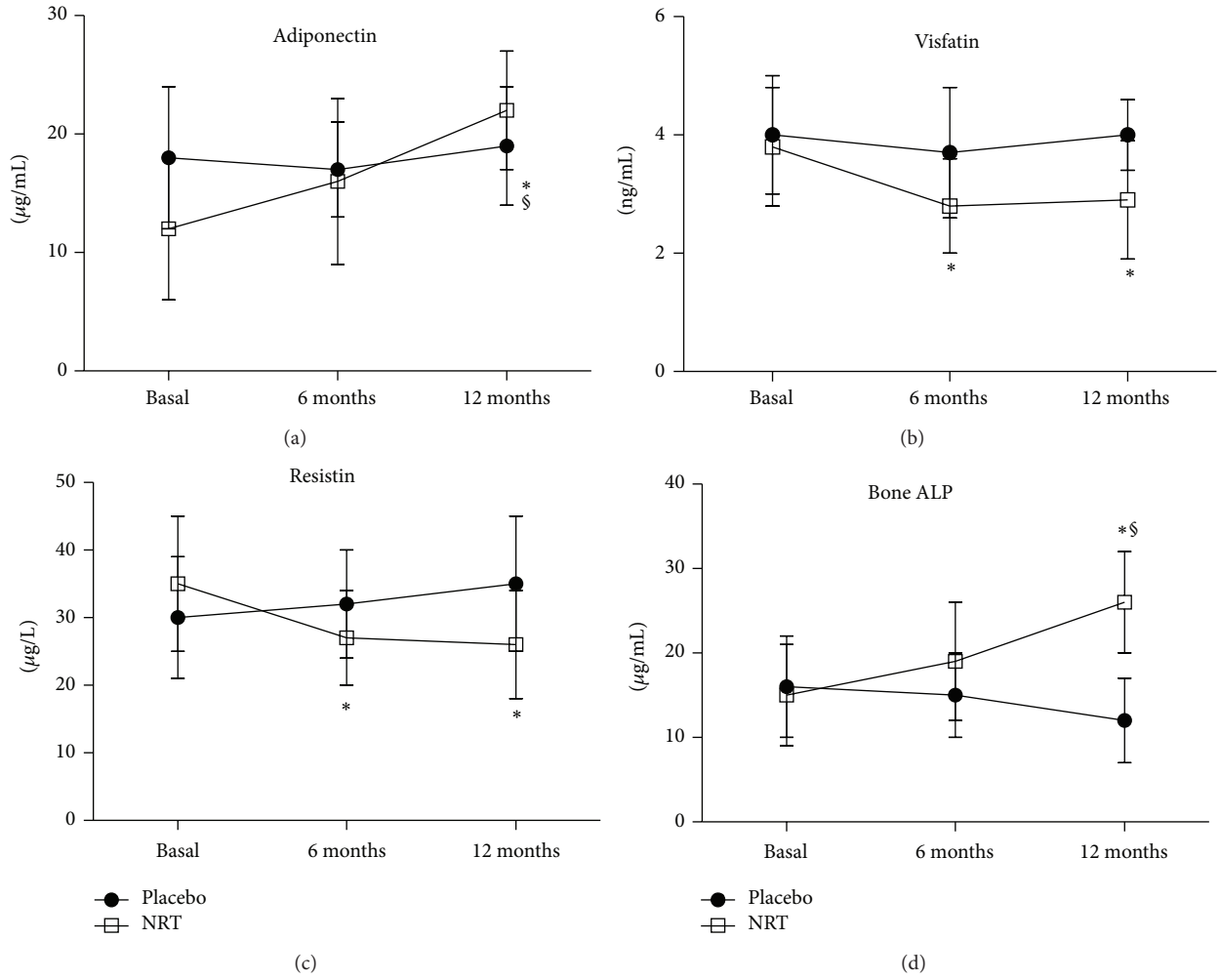

Figure 3: Adiponectin, visfatin, resistin, and bone-ALP blood levels through the study. $^{*}P < 0.05$ versus basal; $^{\S}P < 0.05$ versus placebo.

b-ALP, visfatin, and resistin, also adiponectin levels were significantly changed in NRT group, compared to placebo (Table 2 and Figures 2 and 3).

The intra-group analysis revealed that only HDL-C and adiponectin were not affected by NRT treatment at the end of the 6 months, while all the other studied variables were significantly changed from basal values (Table 2 and Figures 2 and 3). In the placebo group after 6 months a significant difference from basal values was observed for triglycerides and adiponectin, probably due to diet, despite no changes in BMI were noted in the enrolled subjects (Table 2 and Figures 2 and 3).

4. Discussion

The results of this study demonstrate a protective effect on cardiovascular markers following 6 months administration of a combination of myo-inositol, soy isoflavones, and cocoa polyphenols, in postmenopausal women with metabolic syndrome.

A limit of this study is the number of women enrolled, which reduced the statistical significance of the outcomes; in spite of all that, some interesting results have been obtained. One of these was the persistence, in the six months follow-up period, of the positive results achieved during the six-month treatment period; this data concerns all the markers studied. No differences were highlighted in BMI and waist circumference between and within groups, but this may be due to the short treatment period and somewhat to the low adherence to diet. In the treated group, serum glucose and triglycerides values significantly decreased after six months, maintaining in the subsequent 6 months a significant difference from basal levels. These results are in accordance with other studies, in which each component of the supplement of this trial was used [10, 14, 16]. No difference in HDL-C was shown either between groups or from 6-month to basal values; this data is confirmed by some studies in which only cocoa polyphenols were used [5, 17], but not by others [10, 14, 16], in which a significant difference compared to control group was shown, when each single component of the supplement studied was used. Blood pressure didn't significantly change through the

study period, although significant differences were highlighted in other studies in which either myo-inositol or cocoa polyphenols were used [6, 10]; probably the limited number of women studied has negatively influenced this outcome. More interesting were the data about biomarkers which usually depend on insulin resistance. In particular, serum adiponectin levels were significantly increased, after 12 months, in the treated group compared to the control group and also increased at 12 months from basal values in the same group. This result is in accordance with a recent study of our group where genistein aglycone was used [14]. Adiponectin is an adipocyte-specific, secreted protein that sensitizes the liver and muscle to the action of insulin [18]. It is the only adipocyte-derived hormone to be downregulated in the insulin-resistant state, so the levels of adiponectin strongly correlate with basal insulin levels and insulin sensitivity [19]. This explains why low concentrations of adiponectin are associated with increased prevalence of metabolic syndrome, especially in postmenopausal women [20]. If an improvement of insulin resistance is expeceted using isoflavones, because they act through estrogen receptors; or myo-inositol, because of its insulin-like effect [10, 11]; an insulin sensitizing effect is not yet clear for cocoa polyphenols. According to Grassi and coworkers [21], a possible explanation might be found in the positive effect that cocoa extracts have on endothelium-dependent relaxation; in fact, since insulin sensitivity may be in part considered dependent on NO availability, cocoa polyphenols may improve NO production and insulin sensitivity as a consequence [22]. For resistin and visfatin a significant difference occurred only in the treated group, in which both markers were significantly reduced after 6 months of supplement assumption, reaching a plateau until the 12th month. Resistin, is a peptide hormone produced by adipocytes that is more highly expressed in omental and abdominal subcutaneous white fat [23]. In postmenopausal, and in obese women, resistin levels nearly double, representing the hormone that links obesity to diabetes [24]; in addition, a close association of resistin to metabolic syndrome has been recently established [25]. Visfatin role is controversial, but recent evidences have shown increased serum levels in overweight/obese, type-2 diabetics, metabolic syndrome, and CVD patients [26]. Kim and coworkers [27] have suggested that visfatin may act as the underlying pathophysiological trigger for metabolic syndrome in postmenopausal women and as a marker of abdominal fat deposition and tissue inflammation. The here reported significant reduction in visfatin values, is in agreement with our recent findings in postmenopausal women with metabolic syndrome taking the isoflavone genistein for 12 months [14]. Among the biomarkers evaluated, bone-ALP was also included. This molecule is synthesized by the osteoblasts and it is considered a specific marker of bone formation [28]. In this study, the group receiving the combination formula showed increased levels of bone-ALP over time, with significant differences compared to the placebo group after 12 months and a significant difference with respect to basal values. This result is in accordance with a previous study [29], showing that 54 mg of genistein improved bone turnover, prevented osteoporosis, and increased bone mass density in postmenopausal osteopenic women. Thus, we may hypothesize that probably isoflavones have determined an improvement in bone turnover; however, an experimental study [30] has shown that also myo-inositol is essential for osteogenesis and bone formation.

In conclusion, the supplement used in this study has shown to improve most of the biomarkers linked to metabolic syndrome, suggesting a possible reduction of CVD risk. Furthermore, this study has shown that the positive effects of the supplement may last in the subsequent six months, together with an increase in bone formation. Further studies are warranted to reproduce the present data in a larger cohort of postmenopausal women with metabolic syndrome.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Research Article

The TG/HDL-C Ratio Might Be a Surrogate for Insulin Resistance in Chinese Nonobese Women

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Obejective. To examine the discriminatory power of triglyceride (TG) and triglyceride to high-density lipoprotein cholesterol ratio (TG/HDL-C) for insulin resistance (IR) in a normoglycaemic Chinese population. *Methods*. The data were collected from 711 individuals. The normoglycaemic individuals were eventually included in the study (n = 533, age: 62.8 ± 6.6 years, male: 56.8%), who were with a fasting plasma glucose < 6.1 mmol/L and without a history of diabetes. IR was defined as the upper quintile (≥1.6) of homeostasis model assessment of IR. Area under the receiver operating characteristic curve (AROC) was used to examine the discriminatory power. *Results*. The discriminatory power of TG/HDL-C for IR was acceptable in women with a BMI < 24 kg/m^2 or waist circumference < 80 cm (AROCs: 0.718 and 0.713, resp.); however, the discriminatory power was not acceptable in the obese women. TG/HDL-C was not an acceptable marker of IR in men. The discriminatory power of TG for IR was not acceptable in both men and women. *Conclusions*. The discriminatory power of TG/HDL-C for IR differs by gender and obesity index in the normoglycaemic Chinese population, and TG/HDL-C could discriminate IR in the nonobese and normoglycaemic women.

1. Introduction

Insulin resistance is characterized by a decrease in the ability of insulin to stimulate the use of glucose by muscles and adipose tissues and to suppress hepatic glucose production and output [1]. Insulin resistance plays an important pathogenic role in causation of diabetes and cardiovascular diseases (CVD). Therefore, identification of insulin resistance would facilitate selection of high-risk individuals for primary preventions of these interrelated diseases. Currently, the standard methods of measuring insulin resistance includes the glucose clamp, the insulin suppression test, and the frequently sampled intravenous glucose tolerance test [2–4], but these tests are not routinely measured in most clinical practices owing to the time and cost involved. Some simple methods of measuring insulin resistance includes fasting insulin, fasting plasma glucose (FPG)/fasting insulin (FINS), homeostasis model assessment method of insulin resistance (HOMA-IR), and 1/(FPG × FINS). These methods all include insulin, but plasma insulin is not routinely measured in most clinical laboratories. So, identification of insulin resistance by simple surrogates would be useful in clinical practices.

Recently, some studies [2, 5–7] gave us some hopes, and these studies showed that in patients without diabetes, triglyceride (TG) and triglyceride to high-density lipoprotein cholesterol ratio (TG/HDL-C) were closely and positively related to insulin resistance, and the two variables were recommended as surrogates for insulin resistance. However, not all studies found TG and TG/HDL-C to be associated with insulin resistance. For example, some studies [8–11] showed that TG and TG/HDL-C were not reliable markers of insulin resistance in some populations. These previous results were inconsistent, and further studies are still necessary. On the other hand, these previous studies mainly focused on non-Asian populations. Therefore, the aims of our study were to examine the discriminatory power of TG and TG/HDL-C for insulin resistance in a normoglycaemic Chinese population.

2. Methods

2.1. Study Population. In 2007, a health examination was performed in 711 individuals in an urban community located in Chengdu, Sichuan province, China. The cohort was a part

of a study supported by megaprojects of science research for the 11th five-year plan, China (Trends in the incidence of metabolic syndrome and integrated control in China). The detailed information of the study has been reported elsewhere [12]. We included normoglycaemic individuals in the study, who were with a FPG < 6.1 mmol/L and without a medical history of diabetes [13]. In addition, we excluded individuals using any medication known to influence insulin resistance or lipid metabolism (such as corticosteroids and lipid-lowering drugs). Because estrogen exposure could lead to an elevation of TG levels, women receiving exogenous estrogens also were excluded. Therefore, 533 individuals with complete data (age: 62.8 ± 6.6 years, range: 45.0~83.0, male: 56.8%) were available for analysis. The study was approved by Ministry of Health of China, as well as by the Ethics Committee of West China Hospital of Sichuan University. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. All participants provided written informed consent.

2.2. Data Collection. In 2007, medical professionals conducted a survey of CVD risk factors according to the MONICA protocol. The survey content included standardized questionnaire, physical examination, and laboratory tests. The questionnaire included sex, age and CVD risk factors, such as smoking status, alcohol consumption levels, physical activity, and CVD family history. Physical examination included blood pressure, height, weight, waist circumference, and hip circumference. Laboratory tests included FPG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C, TG, high sensitivity creactive protein (hsCRP), and insulin. Blood was drawn from the antecubital vein in the morning after a 12 h fasting. Fasting serum insulin was measured by radioimmunoassay (XH-6010, Xi'an, China). Fasting glucose, TG, and HDL-C were measured enzymatically using a MODULAR P800 Analyzer (Roche Diagnostics). These chemistries were measured at the laboratory of West China Hospital (Chengdu, China).

2.3. Related Definitions. Insulin resistance was defined by using HOMA-IR, which was calculated as (fasting glucose mmol/L × fasting insulin mU/L)/22.5. Insulin resistant individuals were defined as those who had the highest quintile value of the HOMA-IR (≥1.6), according to the previous studies [14, 15]. Smoking is the average cigarette consumption ≥ one/day. Alcohol intake is the average intake of alcohol ≥ 50 g/day. Those with hypertension were defined as having systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg and/or currently taking antihypertensive medications. Body mass index (BMI) was calculated as weight (in kg)/height (in m^2).

2.4. Statistical Analysis. Data are presented as means ± standard deviation (SD) for normal variables or median + interquartile range for skewed variables. Smoking and alcohol intake were used as dummy variables. Comparisons between groups were performed by independent t test for normally distributed variables and by the nonparametric Mann-Whitney test for skewed variables. Interactions between categorical variables were evaluated with the Pearson χ^2 test. Area under the receiver operating characteristic curve (AROC) was used to examine the discriminatory power of TG and TG/HDL-C for insulin resistance: AROC of 0.5 = no discrimination, 0.7 ≤ AROC < 0.8 = acceptable, 0.8 ≤ AROC < 0.9 = excellent, AROC ≥ 0.9 = outstanding [16]. For statistical analysis, SPSS (version 10.0; SPSS, Chicago, IL) software was used. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Demographic Data. Demographic data are presented in Table 1. Age, waist circumference, waist circumference/hip circumference, prevalence of smoking and alcohol intake, SBP, DBP, and TC/HDL-C were significantly higher in men; however, HDL-C, TG, insulin and HOMA-IR were significantly lower. TG/HDL-C, hsCRP, FPG, LDL-C, BMI, hip circumference, and prevalence of hypertension were similar in both groups. The prevalence of insulin resistance was 20.5% in the population, and the prevalence was higher in women than in men (27.4% versus 15.2%, $P = 0.001$, Table 1).

3.2. Discriminatory Power of TG/HDL-C and TG for Insulin Resistance in Different Population. The correlations between TG and TG/HDL-C with HOMA-IR were not similar in different groups (r for TG and HOMA-IR: all individuals = 0.343, men = 0.316 and women = 0.339; TG/HDL-C and HOMA-IR: all individuals = 0.376, men = 0.360 and women = 0.401; all associations, $P < 0.001$).

For discriminating insulin resistance, TG and TG/HDL-C were not acceptable in the whole population (AROCs: 0.634 and 0.654, resp.). When applied to men or women, AROCs were also not acceptable (men: 0.627 for TG, 0.657 for TG/HDL-C; women: 0.614 for TG, 0.652 for TG/HDL-C, resp.).

When women were divided into different subgroups according to BMI (<24, ≥24 kg/m^2) or waist circumference (<80, ≥80 cm), the discriminatory power of TG/HDL-C for insulin resistance was acceptable in the nonobese women (Table 2; Figures 1(a) and 1(c)). However, the discriminatory power of TG/HDL-C for insulin resistance was not acceptable in the obese women (Table 2; Figures 1(b) and 1(d)). When men were divided into different subgroups according to BMI (<24, ≥24 kg/m^2) or waist circumference (<90, ≥90 cm), the discriminatory power of TG/HDL-C for insulin resistance was not acceptable in each subgroup (Table 2). In addition, TG was not an acceptable marker of insulin resistance in each subgroup (Table 2, Figures 1(a)–1(d)). Further, we categorized the population into different subgroups according to the combination of BMI and waist circumference, and the discriminatory power was not improved, even lower than the single standard of classification (data not shown).

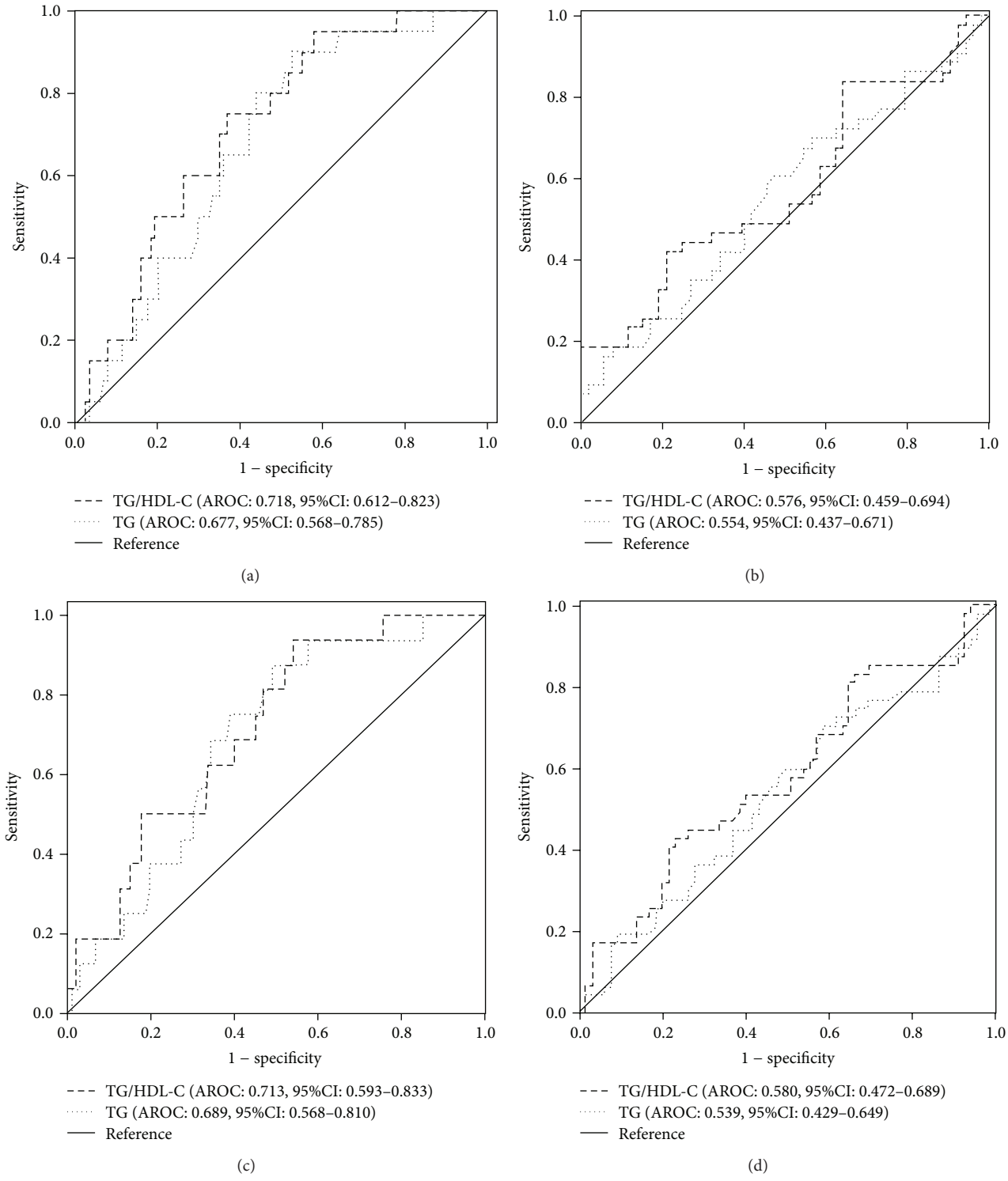

Figure 1: Area under the receiver operating characteristic curves of TG and TG/HDL-C for discriminating insulin resistance in women. AROCs of TG and TG/HDL-C for predicting insulin resistance in women with a BMI < 24 kg/m^2 ((a), $n = 134$) and in women with a BMI ≥ 24 kg/m^2 ((b), $n = 118$). AROCs of TG and TG/HDL-C for predicting insulin resistance in women with a waist circumference < 80 cm ((c), $n = 96$) and in women with a waist circumference ≥ 80 cm ((d), $n = 112$).

Table 1: Characteristics of the individuals.

Variables	Male (n = 303)	Female (n = 230)	P value
Age (years)	63.8 ± 6.2	61.5 ± 6.8	<0.001
BMI (kg/m^2)	23.3 ± 2.9	23.5 ± 3.4	0.512
WC (cm)	83.3 ± 9.3	79.6 ± 9.9	<0.001
HC (cm)	94.8 ± 6.2	94.3 ± 6.9	0.416
WC/HC	0.9 ± 0.1	0.8 ± 0.1	<0.001
SBP (mmHg)	136.3 ± 17.9	131.7 ± 19.9	0.005
DBP (mmHg)	80.5 ± 10.1	78.0 ± 10.1	0.004
Prevalence of hypertension (%)	50.8	45.2	0.199
Smoking (%)	42.2	0.4	<0.001
Alcohol intake (%)	22.8	0.4	<0.001
TC (mmol/L)	4.7 ± 0.8	5.1 ± 0.8	<0.001
LDL-C (mmol/L)	3.0 ± 0.8	3.0 ± 0.7	0.868
HDL-C (mmol/L)	1.4 (1.2, 1.6)	1.6 ± 0.3	<0.001
TG (mmol/L)	1.4 (1.1, 2.0)	1.7 (1.2, 2.2)	<0.001
TG/HDL-C	1.0 (0.7, 1.5)	1.1 (0.8, 1.5)	0.467
TC/HDL-C	3.5 ± 0.7	3.3 ± 0.6	0.003
hsCRP (mg/L)	0.9 (0.4, 2.1)	1.0 (0.5, 2.2)	0.359
FPG (mmol/L)	4.5 ± 0.7	4.6 ± 0.6	0.218
Insulin (mU/L)	4.7 (3.4, 6.6)	5.5 (4.0, 7.9)	<0.001
HOMA-IR	1.0 (0.7, 1.4)	1.1 (0.8, 1.6)	<0.001
Prevalence of insulin resistance (%)	15.2	27.4	0.001

Data are presented as means ± SD, median (interquartile range), or percentage. BMI: body mass index; WC: waist circumference; HC: hip circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol ratio; TG: triglyceride; hsCRP: high sensitivity creactive protein; FPG: fasting plasma glucose; HOMA-IR: homeostasis model assessment method of insulin resistance.

Table 2: Area under receiver operating characteristic curves for discriminating insulin resistance in different subgroups according to body mass index or waist circumference.

Variable	Male (n) (AROC, 95% CI)		Female (n) (AROC, 95% CI)		Male and female (n) (AROC, 95% CI)	
	TG	TG/HDL-C	TG	TG/HDL-C	TG	TG/HDL-C
BMI (kg/m^2)						
<24	0.473 (n = 173) (0.274–0.671)	0.498 (n = 173) (0.293–0.703)	0.677 (n = 134) (0.568–0.785)	0.718 (n = 134) (0.612–0.823)	0.626 (n = 307) (0.521–0.731)	0.645 (n = 307) (0.542–0.749)
≥24	0.643 (n = 130) (0.537–0.748)	0.651 (n = 130) (0.549–0.752)	0.554 (n = 96) (0.437–0.671)	0.576 (n = 96) (0.459–0.694)	0.607 (n = 226) (0.530–0.685)	0.606 (n = 226) (0.528–0.684)
WC (cm)						
A Groups	0.595 (n = 230) (0.457–0.734)	0.632 (n = 230) (0.501–0.763)	0.689 (n = 118) (0.568–0.810)	0.713 (n = 118) (0.593–0.833)	N/A	N/A
B Groups	0.564 (n = 73) (0.424–0.703)	0.575 (n = 73) (0.436–0.714)	0.539 (n = 112) (0.429–0.649)	0.580 (n = 112) (0.472–0.689)	N/A	N/A

AROC: area under receiver operating characteristic curve; CI: confidence interval; BMI: body mass index; WC: Waist circumference; HDL-C: high-density lipoprotein cholesterol ratio; TG: triglyceride; A Groups: <90 cm for men, <80 cm for women; B Groups: ≥90 cm for men, ≥80 cm for women.

3.3. Other Potential Markers of Insulin Resistance. Table 3 shows AROCs for some other potential markers of insulin resistance. For men, the best surrogate for insulin resistance was waist circumference, followed by waist circumference/hip circumference, BMI, and hip circumference (Table 3). For women, BMI was the best surrogate for insulin resistance, followed by waist circumference, waist circumference/hip circumference, and hip circumference (Table 3). AROCs of all clinical variables were acceptable, and AROCs of all biological variables were not acceptable (Table 3).

4. Discussion

Our findings showed that the discriminatory power of TG/HDL-C for insulin resistance differs by gender and

Table 3: Area under receiver operating characteristic curves for potential markers of insulin resistance.

Predicting variables	Male (AROC, 95% CI)	Female (AROC, 95% CI)
Clinical variables		
WC (cm)	0.793 (0.726–0.861)	0.766 (0.700–0.831)
HC (cm)	0.726 (0.649–0.804)	0.714 (0.641–0.787)
BMI (kg/m^2)	0.746 (0.671–0.821)	0.772 (0.707–0.837)
Waist/hip	0.752 (0.678–0.827)	0.723 (0.655–0.791)
Biological variables		
TC (mmol/L)	0.489 (0.403–0.575)	0.411 (0.324–0.498)
HDL-C (mmol/L)	0.333 (0.252–0.414)	0.305 (0.229–0.382)
LDL-C (mmol/L)	0.553 (0.471–0.635)	0.534 (0.447–0.621)
TC/HDL-C	0.651 (0.569–0.734)	0.623 (0.545–0.702)
hsCRP (mg/L)	0.601 (0.514–0.689)	0.688 (0.612–0.764)

BMI: body mass index; WC: waist circumference; HC: hip circumference; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol ratio; hsCRP: high sensitivity creactive protein; AROC: area under receiver operating characteristic curves; CI: confidence interval.

obesity index in normoglycaemic Chinese population, and the discriminatory power of TG for insulin resistance is not acceptable in both men and women. Its presence has been demonstrated in two ways. First, the discriminatory power of TG/HDL-C for insulin resistance was acceptable in the nonobese women (all AROCs > 0.700 in each subgroup). However, TG/HDL-C could not discriminate insulin resistance in the obese women, and in men (all AROCs < 0.700 in each subgroup). Second, the discriminatory power of TG for insulin resistance is not acceptable in both men and women (all AROCs < 0.700 in each subgroup).

The study showed that there were more significant associations between insulin resistance and TG, as well as TG/HDL-C, in women than in men, the same as a previous study [17]. While, Masharani et al. [18] had a different result: in women, there was no significant association between insulin resistance and TG. The current inconsistent results might be caused by sexual and racial differences in lipid profiles. Usually, women show a more favorable metabolic risk profile than men, including lower TG and higher HDL-C levels [19]. Després et al. [20] have also shown that in African Americans and white women, lipoprotein lipase (LPL) activity, which is responsible for clearing TG-containing lipoproteins from the circulation, was higher and this might induce a lower TG levels, and then further causing a weak association between TG levels and insulin resistance in those population. Along with these characteristics, some studies have shown that TG and TG/HDL-C were not reliable markers of insulin resistance in African Americans [8, 9, 11]. However, our study did not draw similar conclusions in Chinese women, and further studies should be warranted. Although sexual and racial differences might influence the associations between insulin resistance and TG, as well as TG/HDL-C, many studies have shown that increasing TG and decreasing HDL-C could deteriorate insulin sensitivity. When TG persists at high levels, heparin activates lipoprotein lipase to increase intravascular lipolysis of circulating TG, thus increasing tissue exposure to free fatty acids (FFA). High FFA may deteriorate insulin sensitivity though oxidative stress pathway [21, 22]. On the other hand, oxidation and inflammation could cause insulin resistance. Since HDL-C has the ability of anti-oxidation and anti-inflammation, decreasing HDL-C might lead to insulin resistance.

Although TG/HDL-C could discriminate insulin resistance in nonobese women, it couldn't work in obese women. A study [23] also showed that the association of TG/HDL-C with insulin resistance was stronger among people with a BMI < $25\,kg/m^2$ than those with a BMI ≥ $30\,kg/m^2$. Some studies [8, 10] also showed TG/HDL-C was not a reliable marker of insulin resistance in the obese. These current data might suggest that TG/HDL-C is most clinically useful for the discrimination of insulin resistance in individuals with normal weight. However, McLaughlin et al. [2] showed TG/HDL-C could discriminate insulin resistance in the subjects with a BMI ≥ $25\,kg/m^2$. Current studies have different results, and confirmatory studies might be warranted. In the present study, all clinical variables were acceptable markers of insulin resistance (Table 3), and those should be recommended to be used in most clinical practices. However, all biological variables were not acceptable (Table 3).

Insulin resistance expressed by HOMA-IR is generally accepted as a valid method in epidemiological surveys. However, there is hardly any consensus on the cut-off points. Values based on 50th percentile [24, 25], 75th percentile [26], 90th percentile [27], lower boundary of the top quintile [14, 15], or tertile [2, 3] of HOMA-IR have been used previously. We defined insulin resistance as HOMA-IR greater than the 80th percentile (≥1.60), which was commonly practiced [14, 15]. In addition, when we performed a separate analyses with insulin resistance defined by the top quartile of HOMA (>1.47) or 90th percentile (>2.02), we obtained similar results (data not shown). Further, insulin resistance is reported to occur at HOMA levels that range from 2.00 to 4.00 in non-Asians, even greater [28, 29], and the threshold levels are lower in Asians, from 1.38 to 2.00 [14, 30, 31]. Our HOMA threshold of 1.6 is within this range. Although we defined insulin resistance a little arbitrarily, it might be accepted in clinical practices.

The study also had several limitations. Firstly, the major limitation of our study was failure to use a glucose clamp,

an insulin suppression test, or a frequently sampled intravenous glucose tolerance test. However, those methods for all individuals over the course of our study were not feasible for pragmatic reasons and logistics. Secondly, the absence of an oral glucose tolerance test might miss some patients with diabetes. Thirdly, because of the relatively small sample size, the results of our study might have limited statistical power. No comparisons between different races might be another limitation.

5. Conclusion

In conclusion, the discriminatory power of TG/HDL-C for insulin resistance differs by gender and obesity index in the normoglycaemic Chinese population, and the discriminatory power of TG for insulin resistance is not acceptable in both men and women. TG/HDL-C could discriminate insulin resistance in the nonobese and normoglycaemic women, and it should be recommended in clinical practices. TG might not be recommended for clinical practices. Further studies should include different ethnic backgrounds, and the gold standard test for evaluating insulin resistance should be used.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publishing of this paper.

Authors' Contribution

Jiyun He and Sen He equally contributed to the paper.

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Review Article

Coronary Heart Disease in Postmenopausal Women with Type II Diabetes Mellitus and the Impact of Estrogen Replacement Therapy: A Narrative Review

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Coronary heart disease is the main cause of death in postmenopausal women (PMW); moreover its mortality exceeds those for breast cancer in women at all ages. Type II diabetes mellitus is a major cardiovascular risk factor and there is some evidence that the risk conferred by diabetes is greater in women than in men. It was established that the deficiency of endogenous estrogens promotes the atherosclerosis process. However, the impact of estrogen replacement therapy (ERT) on cardiovascular prevention remains controversial. Some authors strongly recommend it, whereas others revealed a concerning trend toward harm. This review tries to underlines the different components of cardiovascular risk in diabetic PMW and to define the place of ERT.

1. Introduction

The mortality attributable to coronary artery disease (CAD) exceeds breast cancer mortality in women at all ages [1]; however CAD is the main cause of death in postmenopausal women (PMW) [2]. It is beyond doubt that the cardiovascular risk is multifactorial and several factors come into play, but such a difference has been attributed to the protective effects of female sex hormones, particularly estrogens, before menopause [3]. Type II diabetes mellitus (DM) is a major risk factor for myocardial infarction (MI) and CAD [4–6]. There is some evidence that the risk conferred by DM is greater in women than in men [7]; indeed, in a 13-year prospective study, the incidence of major cardiovascular events in subjects without DM was roughly sixfold greater in men than in women. In presence of DM, the gender difference was lost [8]. Literature about the impact of estrogen replacement therapy (ERT) on the cardiovascular risk is controversial. Some authors supported a protective cardiovascular benefit of ERT after menopause [9], while randomized placebo-controlled trials carried out in both primary [10] and secondary [11] preventions showed a concerning trend toward harm. For all these reasons many questions remain unanswered. This review tries to underline the different components of cardiovascular risk in diabetic PMW and to define the place of ERT.

2. Cardiovascular Risk in Postmenopausal Women

With age, women become more likely to develop type 2 DM: at the age of 50–59 years, approximately 12.5% of women have a known type 2 DM; at the age of 60 years and older, the rate increases to 17-18% (a 25–30% increase). Moreover, type 2 DM remains undiagnosed in more than one-third of these women [12]. Diabetic PMW are three times more likely to develop CAD or stroke than nondiabetic women [13–15]. Furthermore, a diabetic woman is four times more likely to die from MI than a diabetic man [16].

The increased rate of CAD in PMW seems related, in part, to the loss of the protection offered by endogenous estrogen. This finding is supported by the dramatic increase in CAD seen in women after surgically induced menopause [17]. On the other hand, a greater incidence of hypertension and hyperlipidemia as well as an elevated body mass index is observed after menopause [18].

With age, the body of a PMW tends to lose lean body tissue and gain in adipose tissue, particularly in abdominal location [19]. The sedentary lifestyle that often accompanies aging may also contribute to obesity. As a consequence, the insulin resistance increases with its linked dyslipidemia and coagulation abnormalities [19]. In fact, the insulin resistance state due to DM is responsible for an increased hepatic synthesis of triglyceride- (TG-) rich lipoproteins and a faster clearance of high-density lipoproteins cholesterol (HDL-C) [20–22]. Therefore, the dyslipidemia in postmenopausal diabetic women is characterized by elevated plasma TG, reduced HDL-C, and elevated small low-density lipoprotein (LDL) serum levels [23]. Abnormalities in coagulation and fibrinolysis are often seen in type 2 DM including cardiovascular risk indicators such as fibrinogen, factor VII, von Willebrand factor, tissue type-plasminogen activator antigen, and plasminogen activator inhibitor-1 (PAI-1) antigen and activity [24–27]. In addition, peri- and postmenopausal increase in coagulation [28] and decrease in fibrinolysis [29] have been described. Although it has been shown that premenopausal women produce significantly less thromboxane B2 than age-matched men, women show a linear increase in the level of the prostaglandin during the postmenopausal years, whereas such an increase has not been found in men [30].

Menopause is associated with an increase in blood pressure (BP) and a decrease in physiologic nocturnal BP fall [31]. Furthermore, diabetic subjects have increased vascular load and abnormal 24 h BP profiles [32]. These factors may play a role in the increased risk of cardiovascular events in diabetic PMW.

Approximately 25% of PMW smoke cigarettes [33]. Cigarette smoking is associated highly with cardiovascular disease, and in women, it is estimated that 21% of all mortality from cardiovascular disease is related to cigarette smoking [33]. Oncken et al. [34] found that smoking cessation in PMW decreases systolic BP by 3.6 ± 1.9 mm Hg and awake heart rate by 7 ± 1 beats/min. These hemodynamic changes are due in part to reductions in sympathetic nervous system activity [34].

DM is also associated with a diminished nitric oxide bioavailability This promotes atherogenesis through decreased leukocyte adhesion, increased platelet aggregation, and increased vascular smooth muscle growth [35]. This also can cause constriction of coronary arteries during physical or emotional stress, contributing to myocardial ischemia [36]. Women may have false-positive treadmill electrocardiographs with normal coronary angiograms. The so-called syndrome X combining typical angina, ST segment depression, and normal coronary angiography is much more common in women with estrogen deficiency [37, 38]. Compared with men, women had more symptoms and less anatomic coronary artery disease at baseline, with persistence of higher angina rates with or without prompt revascularization [39].

In a retrospective analysis of the Women's Angiographic Vitamin and Estrogen (WAVE) trial, a multicenter randomized trial on progression of atherosclerosis in PMW, Ahmad et al. [40] found a complex relation between DM and the progression of CAD in PMW. In fact, clinically apparent DM, not elevated glycosylated hemoglobin (HbA1c) alone, appears to promote the progression of established coronary lesions even in low HbA1c rates. This raises the possibility that coronary narrowing of existing stenosis in diabetic women may be due to negative remodeling, a complex process that might be less dependent on hyperglycemia than new lesion formation. There has been considerable interest regarding the importance of sex in contributing to mortality rate after percutaneous coronary revascularization. The 1985-1986 National Heart, Lung, and Blood Institute's Coronary Angioplasty Registry documented a hospital mortality rate of 2.6% in women versus 0.3% in men. Some of the difference was related to the fact that women were older and had a higher risk: congestive heart failure, diabetes, and multivessel disease. However, even after adjusting for these parameters, women had a significantly higher mortality rate [41]. Bell et al. reported the same results in the Mayo Clinic experience with 3557 coronary interventions from 1979 to 1990 [42]. Despite the improvement in the management of MI and complications [43, 44], the long-term prognosis remains unsatisfactory in this subsets of patients, particularly diabetics [45, 46]. In fact, DM is associated with impaired perfusion and distal embolization, which contribute to explaining the higher mortality [47]. Therefore, it is difficult to predict the prognosis of percutaneous revascularization in diabetic PMW. Complete revascularization, when possible, is recommended [48]. Second generation of drug-eluting stents is generally preferred [49] and it was shown that diabetic women required larger stents than diabetic men [50]. It is notable that coronary artery bypass surgery in women is more cumbersome in comparison with males, requiring longer intubation times, intensive care unit length of stay, and hospital length of stay [51]. Arterial grafts are recommended. Indeed, the use of a radial artery graft has been proven to improve survival compared with use of a saphenous vein graft [52].

3. Cardiovascular Effects of Estrogen Replacement Therapy

Estrogen has favorable impact on the risk factors of atherosclerosis and therefore CAD [53]. The inflammatory process in atherosclerosis involves a large group of factors and molecules [54]. Growth factors and cytokines play a central role in the development of atherosclerosis [55]. The presence of estrogen receptors, found on human monocytes, suggests that estrogen may modulate the release of such molecules [56]. Patients who had undergone a complete hysterectomy showed higher levels of interleukin 1 (IL-1) activity than postmenopausal women who were under hormone replacement therapy [57]. Aune et al. found that

after 12 months of a hormone replacement therapy (HRT), the levels of tumor necrosis factor (TNFα) produced by lipopolysaccharide-stimulated macrophages had decreased significantly in both patients receiving estrogen orally and those receiving treatment transdermally [58]. By studying the levels of cytokines in postmenopausal women on HRT and those who were not on a therapy, Kamada et al. [59] found that the women on HRT had a significant increase in colony-stimulating factor, which is known to decrease serum cholesterol.

Although there is some concern over its tendency to increase TG serum levels [60], it was also well established that estrogen administration reduces levels of LDL and increases HDL in PMW, thus restoring the lipid profile back to premenopause state [61]. In rabbit aortas, estrogen therapy was also able to decrease collagen production reducing the progression of atherosclerotic plaque [62]. Many studies have been focused on the relation between estrogen, blood coagulation, and the formation of emboli [63, 64]. Estrogen has two completely opposing effects: proinflammatory effect with D-dimer, metalloproteinase 9 and factor VII and anti-inflammatory effect found with fibrinogen, endothelial adhesion molecules, and plasminogen activator inhibitor-1 (PAI-1) [65, 66]. It is difficult to determine which effect will prevail.

Koh et al. studied the effects of HRT on the levels of PAI-1 (a potent inhibitor of fibrinolysis) and D-dimer (a by-product of fibrinolysis) [66]. D-dimer levels increased proportionately with decreasing PAI-1 levels. These findings showed the impact of estrogen on promoting fibrinolysis. In contrast, it has been demonstrated that estrogen also activates coagulation system [66]. By the administration of estrogen to healthy PMW, Caine et al. [67] noted a dose-dependent increase in thrombin generation and fibrinopeptide A. Furthermore, Scarabin et al. [68] found that an oral regimen of estrogen with cyclic progesterone increased levels of prothrombin fragments 1 and 2 and decreased antithrombin activity in healthy PMW. Lee et al. [69] have shown that the basal endothelium dependent vascular reactivity was significantly decreased in PMW with diabetes compared with normal PMW. Although estrogen supplementation increased endothelium dependent vasodilation not only in PMW with diabetes but also in normal PMW, the endothelial dysfunction was not entirely corrected. The etiology of vascular dysfunction in DM is still not fully understood. High levels of glucose may result in a dysregulation of endothelial nitric oxide synthase enzyme function responsible for a decrease in nitric oxide production [70].

Several hypotheses of the mechanism of vasodilation after estrogen treatment exist including the release of prostaglandin and nitric oxide and the activation of potassium or calcium channels [71–73]. However, such findings were not confirmed by Koh et al. [74] who found that the effects of estrogen on endothelial, vascular dilatory and other homeostatic functions were less apparent in type II diabetic postmenopausal women.

Otherwise, estrogen has also been shown to have some protective capabilities against ischemia-reperfusion injury in various organs. Squadrito et al. [75] exposed rats under 17 β-estradiol regimen and untreated rats to 1 h of left coronary artery occlusion followed by 1 h of reperfusion and found that the administration of estrogen 5 min after the induction of injury decreased the markers and the degree of necrosis. This may be due to the antioxidant effect of estrogen [76]. Indeed, Rifici and Khachadurian [77] described a capital role of 17 β-estradiol in the inhibition of LDL oxidation.

It has been reported that HRT decreases angiotensin converting enzyme activity, which may be one of the factors protecting against CAD. Sumino et al. [78] reported an increased level of bradykinin not only in hypertensive but also in nonhypertensive PMW. It has been also demonstrated that estrogen has calcium channel blocking effects [79, 80]. All these arguments are in favor of an antihypertensive role of estrogen. However, Hayward et al. [32] did not find any beneficial effect of HRT on indexes of arterial load and ambulatory BP in diabetic postmenopausal women.

4. Place of the Hormone Replacement Therapy in Cardiovascular Prevention in Postmenopausal Women

It is difficult to predict the overall harm or benefit of HRT on the cardiovascular system considering the pleiotropic effects of estrogens due to the ubiquitous distribution of estrogen receptors in different organs and systems. In both Heart and Estrogen/Progestin Replacement Study (HERS) [11] and Women's Health Initiative (WHI) [10] trial, the increased risk of vascular and thromboembolic events was observed particularly when starting HRT. In WHI, this risk increased mostly within the 2 first years of HRT [10]. In HERS, the relative risk for coronary events was increased more than twofold within the first 4 months and also normalized within 2 years [11]. Otherwise, the effects of estrogen may vastly vary depending on the age, the other risk factors, and the stage of CAD [81]. As the risk of myocardial infarction increases sharply not before the late fifties followed by cerebrovascular disease a decade later, the results of neither HERS nor WHI can be in part transferred to the HRT prescription in peri- and early menopause. A report on more than 24,000 diabetic postmenopausal women revealed an increased rate of MI within the first year of HRT, only in women with a previous MI [82]. However to conclude that WHI only comprises healthy women is inadequate, since many women at the age of 60 will have silent ischaemia, especially those with DM. Table 1 summarized the findings of some key studies focusing on the HRT impact on cardiovascular risk in PMW.

The administrated dose of estrogen has also a determining influence. In fact, in the Northern California Kaiser Permanente Diabetes, the most important decrease of cardiovascular risk was obtained with 0.3 mg conjugated estrogens or 2 mg ethinyl estradiol. With higher doses, this reduction vanished and even an elevated risk appeared [82].

In addition, it was clearly demonstrated that socioeconomic status, correlated with the use of HRT, influences the cardiovascular risk in postmenopausal women [83]. The characteristics of the women using HRT differ from those of the nonusers. Indeed, HRT users tend to have greater contact with the health care system. This issue is particularly capital

Table 1: Summary and conclusions of some key studies focusing on the hormonal replacement therapy impact on cardiovascular risk in postmenopausal women.

Study	Year	Design of the study	*N* patients	HRT	Follow-up	Conclusions
Grodstein et al. [9]	1996	Prospective study	59,337	ERT alone or combined HRT	16 years	Current hormone users, regardless of whether they used ERT alone or combined HRT, tended to have a better risk profile than women who had never used HRT.
Hulley et al. [11] (HERS trial)	1998	Randomized, blinded, placebo-controlled secondary prevention	2,763	0.625 mg of conjugated equine oestrogens + 2.5 mg medroxyprogesterone acetate	4.1 years	HRT did not reduce the overall rate of CAD events in PMW with established CAD. Moreover, HRT did increase the rate of thromboembolic events and gallbladder disease.
Rossouw et al. [10] (WHI trial)	2002	Randomized placebo-controlled primary prevention trial	16,708	0.625 mg of conjugated equine oestrogens + 2.5 mg medroxyprogesterone acetate	5.2 years	Overall health risks exceeded benefits from use of HRT in healthy PMW. HRT should not be indicated for CAD primary prevention.
Ferrara et al. [82] (Northern California Kaiser Permanente Diabetes Registry)	2003	Survey follow-up study	15,435	ERT alone or combined HRT	3 years	In diabetic PMW without a recent MI, use of combined HRT was associated with decreased risk of MI. However, HRT was associated with increased risk of MI in women with history of a recent MI.

CAD: coronary artery disease; ERT: estrogen replacement therapy; HRT: hormonal replacement therapy; MI: myocardial infarction; PMW: postmenopausal women.

in diabetics. For example, a diabetic PMW under HRT may have more chance to be better followed for her DM and earlier diagnosed for nephropathy, retinopathy, and CAD.

5. Conclusion

The incidence and the mortality of CAD in women increase after menopause. The loss of ovarian function and the subsequent deficiency of endogenous estrogens, added to age, abdominal obesity and particularly DM, promote the atherosclerosis process. It is well established that estrogen has favorable effects on some of the major risk factors of CAD. However, only large clinical trials may help to decide which dose, for which women, and at which age HRT is beneficial or harmful. Finally, cardiovascular prevention requires correcting the lifestyle and other classical risk factors, particularly a strict control of diabetes, since estrogen alone cannot be expected to counteract the entire cardiovascular risk.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Fasting Hyperglycemia Increases In-Hospital Mortality Risk in Nondiabetic Female Patients with Acute Myocardial Infarction: A Retrospective Study

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Previous studies had shown that elevated admission plasma glucose (APG) could increase mortality rate and serious complications of acute myocardial infarction (AMI), but whether fasting plasma glucose (FPG) had the same role remains controversial. In this retrospective study, 253 cases of AMI patients were divided into diabetic ($n = 87$) and nondiabetic group ($n = 166$). Our results showed that: compared with the nondiabetic patients, diabetic patients had higher APG, FPG, higher plasma triglyceride, higher rates of painless AMI ($P < 0.01$), non-ST-segment elevation myocardial infarction (NSTEMI), and reinfraction ($P < 0.05$). They also had lower high density lipoprotein cholesterol and rate of malignant arrhythmia, but in-hospital mortality rate did not differ significantly ($P > 0.05$). While nondiabetic patients were subgrouped in terms of APG and FPG (cut points were 11.1 mmol/L and 7.0 mmol/L, resp.), the mortality rate had significant difference ($P < 0.01$), whereas glucose level lost significance in diabetic group. Multivariate logistic regression analysis showed that FPG (OR: 2.014; 95% confidence interval: 1.296–3.131; $p < 0.01$) but not APG was independent predictor of in-hospital mortality for nondiabetic patients. These results indicate that FPG can be an independent predictor for mortality in nondiabetic female patients with AMI.

1. Introduction

Incidence of AMI in female patients is increasing year by year after menopause, especially for type 2 diabetes mellitus (T2DM) patients. Gender disparity in clinical outcome of AMI patients with or without T2DM is still elusive. Women with AMI are more inclined to gain a poorer outcome than men [1–3]. Plasma glucose is often considered as an important predictor of mortality after AMI [3–6]. But the impact of fasting plasma glucose (FPG) on early mortality and serious cardiovascular complications such as malignant arrhythmia, cardiac shock, heart failure, and reinfarction remains unclear.

Plasma glucose level in the acute phase of AMI is closely related to in-hospital mortality rate and serious complications of AMI. Previous studies have demonstrated that there is a near-linear positive relationship between admission plasma glucose (APG) or HbA1c and in-hospital mortality in diabetic and nondiabetic AMI patients [7, 8]. However, other studies showed that the relationship is u-shaped in AMI patients [9–11]. Moreover, previous studies mainly focused on the relationship between APG and clinical outcome. Limited data is available for association between FPG and clinical outcome of AMI with or without T2DM in female patients.

To assess the relationship between FPG and AMI prognosis in female patients, we conducted this retrospective analysis to determine the association between APG, FPG, and serious cardiovascular complications of female AMI patients with or without diabetes.

2. Patients and Methods

2.1. Subjects and Diagnostic Criteria. From January 2002 to February 2014, a total of 253 cases of consecutive female patients who were admitted to the Fifth Affiliated Hospital of Sun Yat-sen University in China with their first AMI

diagnosis were enrolled into the retrospective study. AMI was defined by the following characteristics: chest pain consistent with ongoing myocardial ischemia persisting >30 minutes, ischemic electrocardiographic changes, and positive biochemical cardiac necrosis markers measurement (peak creatinine kinase value >2 times the normal upper limit or elevation of serum troponin I(cTnI) or serum troponin T(cTnT)). STEMI was diagnosed if ST-segment elevation ≥1 mm occurred in ≥1 lead or new left bundle branch block (LBBB) was found in ECG with biochemical evidence of myocardial necrosis. NSTEMI was diagnosed in patients with ≥1 positive biochemical cardiac necrosis markers measurement without new ST-segment elevation in ECG. Malignant arrhythmia is defined as fast or slow arrhythmia that significantly influences blood flow dynamics, including ventricular tachycardia, ventricular flutter, ventricular fibrillation, three-degree AVB, and fast atrial fibrillation with unstable hemodynamic. Cardiogenic shock was defined as reduced blood pressure (SBP < 90 mmHg or a drop of mean arterial pressure > 30 mmHg) and/or low urine output (<0.5 mL/kg/h), with a pulse rate > 60 bpm with or without evidence of organ congestion [12].

Enrolled patients were divided into diabetic group and nondiabetic group based on their final diagnosis [13]. Patients were thought to have diabetes if they had a previous or current diagnosis of diabetes, regardless of glycemic status on admission. The exclusion of T2DM was confirmed by the measurement of nonfasting glucose and fasting glucose before discharge. Each group was divided into two prespecified groups based on APG level (<11.1 and ≥ 11.1 mmol/L) and FPG level (<7.0 and ≥ 7.0 mmol/L). And we defined the former (APG < 11.1 mmol/L and FPG < 7.0 mmol/L) as nonhyperglycemia subgroup and the latter as hyperglycemia subgroup. This study excluded patients with a history of malignant tumor, chronic renal failure (creatinine > 451 μmol/L), liver cirrhosis, serious infected diseases, and previous myocardial infarction.

2.2. Clinical Data Collection. Clinical symptoms and signs including chest pain or painless, systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were recorded on admission. Blood samples, including APG, creatinine, and creatinine kinase (CK), were measured at the time of hospital admission. FPG, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured in the next day's morning after overnight fasting. According to the results of electrocardiograph (ECG), they were classified into non-ST-segment elevation myocardial infarction (NSTEMI) or ST-segment elevation myocardial infarction (STEMI). Killip class was used for the assessment of the severity of heart failure. The primary end point was all-cause in-hospital mortality; the second end points were serious cardiovascular complications such as malignant arrhythmia, cardiac shock, heart failure, and reinfarction.

2.3. Statistical Analysis. Continuous variables are expressed as mean ± SD or median ± interquartile range, and categorical variables were reported as numbers and percentages. Statistical analysis was performed with chi-square test for categorical variables. The *t*-test was used for continuous variables. Logistic regression analyses were used to determine the predictors of in-hospital mortality. In order to account for the influence of the risk factors on mortality rate, we performed a enter regression analysis with in-hospital death as outcome and the other risk factors (age, APG, FPG, SBP, DBP and HR at admission, blood lipid, and creatinine) as covariates. All 2-sided P values <0.05 were considered statistically significant. Analyses were done using the statistical software SPSS 13.0.

3. Results

3.1. Baseline Clinical Characteristics. From February 2002 to February 2014, a total of 253 female patients with their first AMI diagnosis were enrolled in this study. Diabetic group included 87 patients (34%) and nondiabetic group included 166 patients (66%). They had average ages of 70.11 ± 9.80 and 70.32 ± 12.30 years old, respectively. There was no difference in the age between the two groups, as shown in Figure 2.

Table 1 presents the baseline clinical characteristics of patients with or without diabetes. There was significant difference in APG and FPG between the two groups. Mean APG was significantly higher in diabetic patients than nondiabetic patients (13.9 ± 6.2 versus 8.5 ± 3.7 mmol/L, $P < 0.01$). Besides, mean FPG was also remarkably higher in diabetic patients (9.1 ± 3.5 versus 5.9 ± 1.3 mmol/L, $P < 0.01$). For the blood lipid profile, plasma triglyceride level was obviously higher and HDL-C was lower in diabetic group. Compared with the nondiabetic group, diabetic patients were more likely to have atypical clinical presentations of AMI. The proportions of painless AMI and NSTEMI were statistically higher among diabetic patients than the nondiabetic group. In addition, diabetic patients may have a greater reinfarction rate after the first AMI than nondiabetic patients (13.79% versus 6.02%, $P < 0.05$). However, the incidence rate of malignant arrhythmia in diabetes group was lower than nondiabetic group (2.3% versus 11.45%, $P < 0.05$).

3.2. Relationship of APG and FPG to Serious Complications of AMI. The association between different blood glucose level (APG and FPG) and clinical characteristics and complications of AMI was listed in Tables 2 (two missing values for FPG in non-diabetic group) and 3 (11 missing values for FPG in non-diabetic group and six in diabetic group). A total of 34 deaths (13.44%) occurred during hospital stay in two groups (Table 1). In-hospital mortality of diabetic group did not differ significantly from nondiabetic group (16.09% versus 12.05%, $P = 0.37$). But the mortality rate of nonhyperglycemia subgroup (APG < 11.1 mmol/L, FPG < 7.0 mmol/L) and hyperglycemia subgroup (APG ≥ 11.1 mmol/L, FPG ≥ 7.0 mmol/L) was dramatically different in the nondiabetic group (Tables 2 and 3). There was a tendency towards a much higher in-hospital mortality rate in nondiabetic group with the rising APG level and FPG level (Figure 1). When nondiabetic patients were subgrouped by APG level, the in-hospital mortality rate in nonhyperglycemia subgroup and

Table 1: Baseline characteristics of patients with and without diabetes mellitus.

Variable	Nondiabetic group	Diabetic group	*P* value
Cases	166	87	
Age (years)	70.32 ± 12.30	70.11 ± 9.80	0.88
Hypertensions	98 (59.03%)	61 (70.11%)	0.08
Painless AMI	35 (21.08%)	30 (34.48%)	**0.02**
HR (bpm)	83 ± 23	86 ± 21	0.43
SBP (mmHg)	135 ± 30	140 ± 28	0.19
DBP (mmHg)	82 ± 20	80 ± 14	0.34
APG (mmol/L)	8.50 ± 3.73*	13.90 ± 6.21	**0.00**
FPG (mmol/L)	5.90 ± 1.31#	9.10 ± 3.32##	**0.00**
CK (U/L)	719 ± 573	583 ± 560	0.36
TG (mmol/L)	1.26 ± 0.73	1.67 ± 0.98	**0.00**
TC (mmol/L)	5.20 ± 1.12	5.30 ± 1.40	0.63
HDL-C (mmol/L)	1.20 ± 0.34	1.09 ± 0.29	**0.01**
LDL-C (mmol/L)	3.11 ± 0.91	3.18 ± 1.08	0.63
Creatinine (μmol/L)	92 ± 55	120 ± 106	0.08
NSTEMI	56 (33.73%)	41 (47.12%)	**0.04**
Conservative therapy	106 (63.86%)	63 (72.41%)	0.17
Primary PCI	55 (33.13%)	24 (27.59%)	0.37
Malignant arrhythmia	19 (11.45%)	2 (2.30%)	**0.01**
Cardiac shock	34 (20.48%)	15 (17.24%)	0.54
Killip classes III-IV	51 (30.72%)	33 (37.93%)	0.25
Mortality rate	20 (12.05%)	14 (16.09%)	0.37
Reinfarction rate	6.02%	13.79%	**0.04**
Reinfarction interval (month)	40 ± 21	18 ± 16	0.09

Data were presented as mean ± SD for normally distributed and continuous variables (Age, HR, SBP, DBP, APG, FPG, TC, HDL-C, and LDL-C) or median (IQR) for nonnormally distributed variables (CK, TG, creatinine, and reinfarction interval); categorical variables were reported as numbers and percentages. AMI: acute myocardial infarction; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; APG: admission plasma glucose; FPG: fasting plasma glucose; CK: creatinine kinase; TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; NSTEMI: non-ST-segment elevation myocardial infarction; and PCI: percutaneous coronary intervention.
*Data for 164 patients, #data for 155 patients, and ##data for 81 patients.

Table 2: Comparison of different admission glucose levels between nondiabetic group and diabetic group.

Variable	Nondiabetic group (mmol/L)		*P*	Diabetic group (mmol/L)		*P*
	<11.1	≥11.1		<11.1	≥11.1	
Cases	140	24		27	60	
Mortality rate	11 (7.86%)	7 (29.17%)	**0.006**	44 (14.81%)	10 (16.67%)	1.000
Painless AMI	27 (19.29%)	8 (33.33%)	0.121	6 (22.22%)	24 (40.00%)	0.107
TG	1.26 ± 0.81	1.12 ± 0.76	0.409	1.79 ± 1.21	1.62 ± 1.13	0.533
HDL-C	1.20 ± 0.34	1.20 ± 0.30	0.621	1.01 ± 0.25	1.13 ± 0.30	0.177
Creatinine	83.62 ± 57.20	96.04 ± 36.30	**0.013**	102.53 ± 77.26	97.5 ± 66.27	0.690
NSTEMI	47 (33.57%)	9 (37.50%)	0.708	13 (48.15%)	28 (46.67%)	0.898
Cardiac shock	24 (17.14%)	8 (33.33%)	0.116	3 (11.11%)	12 (20.00%)	0.479
Killip classes (III-IV)	38 (27.14%)	11 (45.83%)	0.065	9 (33.33%)	24 (40.00%)	0.553
Malignant arrhythmia	19 (13.57%)	5 (20.83%)	0.630	0 (0.00%)	2 (1.67%)	1.000

hyperglycemia subgroup was 7.86% and 29.17%, respectively ($P < 0.01$). Likewise, when subgrouped by FPG level, the in-hospital mortality rate was 4.32% and 43.75%, respectively ($P < 0.01$). However, there was no significant difference between nonhyperglycemia subgroup and hyperglycemia subgroup in mortality rate in the diabetic group. Moreover, as shown in Table 3, in the nondiabetic group, the incidence of cardiac shock in hyperglycemia subgroup is much higher than nonhyperglycemia subgroup (12.95% versus 56.25%, $P < 0.001$).

3.3. Predictors of In-Hospital Mortality. In order to analyze which factor was closely associated with in-hospital mortality, we did a multivariate logistic regression analysis to eliminate

Table 3: Comparison of different fasting glucose levels between nondiabetic group and diabetic group.

Variable	Nondiabetic group (mmol/L)		P	Diabetic group (mmol/L)		P
	<7.0	≥7.0		<7.0	≥7.0	
Cases	139	16		28	53	
Mortality	6 (4.32%)	7 (43.75%)	**0.000**	4 (14.29%)	7 (13.21%)	1.000
Painless	29 (20.86%)	3 (18.75%)	1.000	7 (25.00%)	20 (37.74%)	0.248
TG	1.28 ± 0.79	0.98 ± 0.58	0.233	1.74 ± 1.21	1.65 ± 1.06	0.780
HDL-C	1.20 ± 0.34	1.16 ± 0.26	0.792	1.04 ± 0.26	1.12 ± 0.31	0.549
Creatinine	72.50 ± 37.25	63.00 ± 54.00	0.291	98.96 ± 72.25	100.02 ± 71.20	0.923
NSTEMI	48 (34.53%)	6 (37.5%)	0.813	12 (42.86%)	27 (50.94%)	0.488
Cardiac shock	18 (12.95%)	9 (56.25%)	**0.000**	2 (7.14%)	10 (18.87%)	0.278
Killip classes (III-IV)	34 (24.46%)	6 (37.50%)	0.408	11 (39.29%)	18 (33.96%)	0.625
Malignant arrhythmia	17 (12.23%)	3 (18.75%)	0.732	0 (0.00%)	1 (1.89%)	1.000

the influence of confounding factors. Our results showed that independent predictors of in-hospital mortality for nondiabetic patients with AMI were FPG (OR: 2.014; 95% CI: 1.296–3.131, $P < 0.01$) and creatinine (OR: 1.011; 95% CI: 1.004–1.017, $P < 0.01$) (Table 4). However, the predictors of in-hospital mortality in diabetic group were age (OR: 1.160; 95% CI: 1.004–1.342, $P < 0.05$) and creatinine (OR: 1.007; 95% CI: 1.001–1.014, $P < 0.05$) (Table 5).

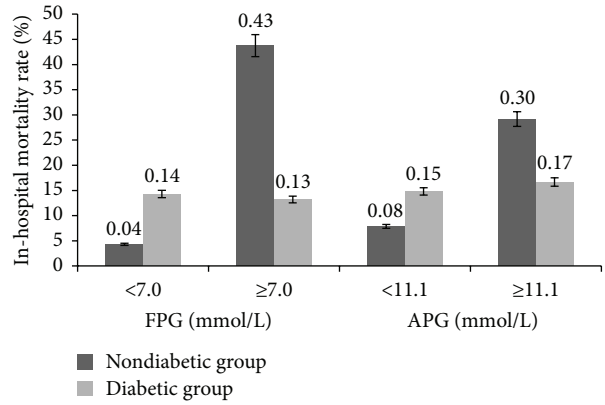

Figure 1: The relationship between blood glucose level and in-hospital mortality rate.

4. Discussion

Several previous studies have demonstrated that elevated APG and HbA1c were powerful predictors of mortality and increased risk of cardiovascular complications in AMI patients both with and without diabetes [13–16]. They found that hyperglycemia was associated with larger infarct size [17], lower left ventricular ejection fraction (LVEF) [18], and poor prognosis. Previous reports also suggested that glycemic status, which poses a risk for CVD, differed in male and female individuals. The reason could be a difference in the basic mechanism of carbohydrate metabolism, hormone, and insulin sensitivity. But there were limited data on FPG and cardiovascular prognoses of female patients with AMI. In the present study, we defined fasting hyperglycemia in acute myocardial infarction phase to be ≥7.0 mmol/L and admission hyperglycemia to be ≥11.1 mmol/L. This glycaemia cutoff point was convergent with other authors' reports [19]. Moreover, this cutoff point was recommended by worldwide standards for a tool for carbohydrate metabolism disorder diagnosis [20]. Our study found that elevated FPG level in nondiabetic group was a strong and independent predictor of increased risk of mortality and cardiac shock in patients with AMI, and elevated APG level was closely associated with higher mortality rate and plasma creatinine level. But in diabetic group, we did not find this association. These results were in accordance with several previous studies including both men and women [21, 22].

In recent years, the importance of FPG in the prognosis of AMI was being gradually recognized. Suleiman et al. [21] reported a higher adjusted prevalence of in-hospital mortality among nondiabetic patients with elevated FPG, using a cutoff value of FPG > 6.1 mmol/L (110 mg/dL) for fasting hyperglycemia. Compared with patients categorized as having normal FPG, the adjusted OR for 30-day mortality progressively increased with higher tertiles of elevated FPG (first tertile: 4.6; 95% CI: 1.7 to 12.7; second tertile: 6.4; 95% CI: 2.5 to 16.6; and third tertile: 11.5; 95% CI: 4.7 to 20.0). Yang et al. [23] reported the FPG-stratified hazard ratios of in-hospital mortality in female patients of 1.037 (95% CI: 0.820–2.262) and 1.174 (95% CI: 0.905–4.432) in mildly hyperglycemic and severely hyperglycemic group after multivariate adjustment. In our present study, we also found the prognostic value of APG in female nondiabetic patients with AMI.

T2DM is already an established risk factor for patients with AMI [24–26]. However, in contrast to these findings, we found higher in-hospital mortality of AMI in nondiabetic patients, contrary to the existing knowledge that the mortality of AMI was common mainly in diabetics. Moreover, our study demonstrated that elevated FPG and APG levels in nondiabetic group were strong and independent predictors of increased risk of mortality with AMI, but, in diabetic group, they were not. It showed that risk factors other than DM had a stronger association with the mortality of AMI in these cases. These results were consistent with the findings in some studies [27, 28]. This phenomenon observed in the

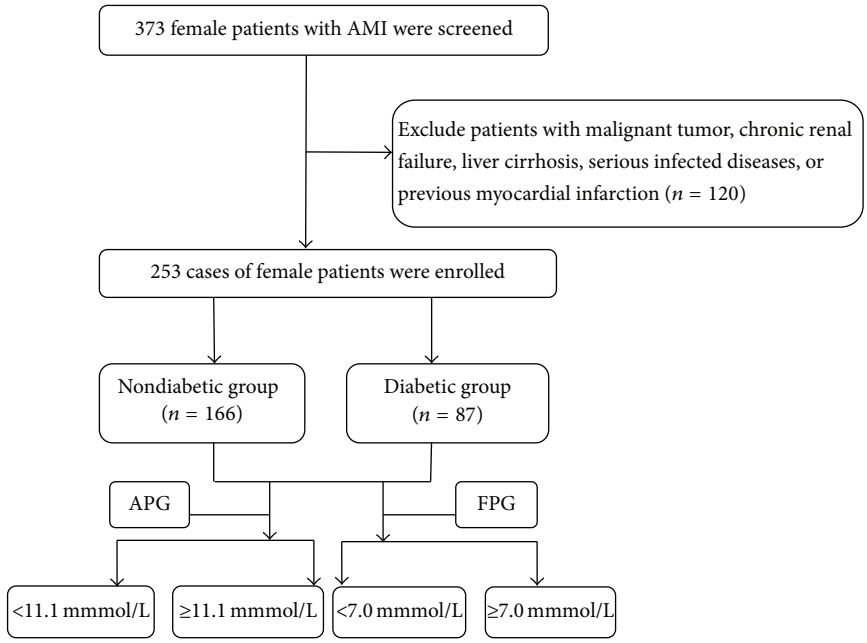

Figure 2: Flow chart of the inclusion of subjects in our study.

Table 4: Logistic regression analysis for mortality rate in nondiabetic group.

Variable	*B*	S.E.	Wald	*P*	OR	95% CI for OR	
						Lower	Upper
Age	0.057	0.031	3.286	0.070	1.059	0.995	1.126
HR	−0.014	0.015	0.834	0.361	0.986	0.957	1.016
SBP	−0.027	0.019	1.999	0.157	0.973	0.937	1.011
DBP	−0.004	0.030	0.020	0.886	0.996	0.938	1.056
APG	0.111	0.082	1.830	0.176	1.117	0.951	1.312
FPG	0.700	0.225	9.672	**0.002**	2.014	1.296	3.131
TG	−0.272	0.605	0.203	0.652	0.762	0.233	2.491
TC	0.050	0.674	0.006	0.940	1.052	0.281	3.939
HDL-C	−1.477	1.464	1.018	0.313	0.228	0.013	4.022
LDL-C	−0.097	0.769	0.016	0.900	0.908	0.201	4.096
CK	0.000	0.000	1.094	0.296	1.000	0.999	1.000
Creatinine	0.010	0.003	10.03	**0.002**	1.011	1.004	1.017

current study may be a result of several factors, including improved treatment in the acute phase of AMI and increased long-term survival resulting from aggressive secondary prevention in diabetic patients [27]. Besides, we found that the frequency of malignant arrhythmia in the nondiabetic group was much higher than diabetes group, suggesting that malignant arrhythmia may contribute to narrowing the gap of short-term mortality between nondiabetic and diabetic patients. However, the underlying mechanisms are still unclear. Previous research indicated that hypoglycemia caused an acquired long QT syndrome and prolonged cardiac repolarization causes fatal cardiac arrhythmias [29]. But it did not fully explain these results. Besides, our study showed that the frequency of myocardial reinfarction was obviously higher in diabetic group, which indicated adverse long-term outcome in diabetic patients. Some studies also found that T2DM may abolish the beneficial effect of primary PCI on long-term risk of clinical reinfarction [30, 31]. For diabetic patients, undergoing primary PCI had the similar reinfarction rate compared with those who received the thrombolysis treatment [31]. Therefore, although T2DM did not impact short-term mortality rate, it still influenced the long-term mortality rate of AMI patients.

The relationship of the cause and effect between hyperglycemia and in-hospital mortality of AMI is still uncertain. On the one hand, serious AMI can cause stress hyperglycemia, resulting from a surge of stress hormones such as adrenaline, noradrenalin, and cortisol which induce or exacerbate an insulin-resistant state [32]. Relative insulin deficiency and excess catecholamine reduced glucose uptake by the ischemic myocardium and promoted lipolysis which increased circulating free fatty acids [21]. On the other hand,

Table 5: Logistic regression analysis for mortality rate in diabetic group.

Variable	*B*	S.E.	Wald	*P*	OR	95% CI for OR	
						Lower	Upper
Age	0.149	0.074	4.044	**0.044**	1.160	1.004	1.342
HR	0.040	0.029	1.913	0.167	1.041	0.983	1.103
SBP	−0.064	0.035	3.422	0.064	0.938	0.876	1.004
DBP	−0.049	0.063	0.592	0.442	0.953	0.842	1.078
APG	0.122	0.090	1.827	0.176	1.129	0.947	1.347
FPG	0.025	0.139	0.032	0.858	1.025	0.781	1.346
TG	1.036	0.778	1.772	0.183	2.818	0.613	12.960
TC	−1.449	1.557	0.866	0.352	0.235	0.011	4.966
HDL-C	−0.237	2.890	0.007	0.935	0.789	0.003	227.501
LDL-C	1.749	1.575	1.233	0.267	5.748	0.262	125.928
CK	0.000	0.000	0.270	0.604	1.000	0.999	1.001
Creatinine	0.007	0.003	4.646	**0.031**	1.007	1.001	1.014

hyperglycemia itself may lead to serious complications of AMI. Induction of endothelial dysfunction, oxidative stress, hypercoagulability, and impaired fibrinolysis may follow after hyperglycemia [21]. These factors ultimately produce vicious cycle. But some animal experiments indicated that short-term hyperglycemia may protest against ischemic myocardium [33, 34]. More researches will need to deeply uncover the mechanisms.

However, there are some reports contradicting our analysis and suggesting that the prognosis of diabetic patients may be significantly poor during the acute myocardial infarction phase. Moriyama et al. [35] presented a 2-fold higher hospital mortality rate in the subgroup of patients with DM and hyperglycemia in comparison to patients with DM without hyperglycemia. Likewise, Scuteri et al. [36] reported higher in-hospital mortality rate in the group of patients with DM and hyperglycemia. Multivariate analysis showed 5-fold risk of death in patients with hyperglycemia over 300 mg/dL and 2.8-fold in patients with hyperglycemia over 218 mg/dL, in comparison to patients with blood glucose level below 161 mg/dL.

In our analysis, the prognosis of patients without DM with concomitant hyperglycemia may, in part, be explained by the following differences in group characteristics. Firstly, patients with hyperglycemia were generally over 60 years, which agreed with a multivariate analysis showing that age was an independent factor of increased mortality rate in 1-year follow-up. In addition, nondiabetic patients with acute hyperglycemia showed an increased rate of inefficient fibrinolysis and the presence of multivessel coronary heart disease. These patients differed not only in the mentioned parameters but also in infarction severity including the concentration of myocardial necrotic markers and left ventricular ejection fraction from normal glucose patients. Hence, the importance of evaluation of plasma glucose during AMI for a better prognosis during follow-up period cannot be disregarded. However, we should realize that Blood glucose value (PFG and APG) is changeable in one day. Normally, the level of blood glucose changes within a limited range in nondiabetic patients. However, the inter- and intra-day glucose variability is relatively high in diabetic patients. In addition, blood glucose level is influenced by many other factors, such as diabetes status, serious complications and treatment. So before we analyse the association between hyperglycemia and complications of AMI, those factors should be took into consideration.

Several limitations of the research should be acknowledged. First, this was a single-center, nonrandomized, and retrospective study with a relatively small number of patients. Second, we did not evaluate long-term outcome of every AMI patient, so the relationship between FPG and long-term prognosis was not exactly assessed.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Guojing Luo and Hong Liu contributed equally to this work.

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Research Article

ATP Synthase β-Chain Overexpression in SR-BI Knockout Mice Increases HDL Uptake and Reduces Plasma HDL Level

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HDL cholesterol is known to be inversely correlated with cardiovascular disease due to its diverse antiatherogenic functions. SR-BI mediates the selective uptake of HDL-C. SR-BI knockout diminishes but does not completely block the transport of HDL; other receptors may be involved. Ectopic ATP synthase β-chain in hepatocytes has been previously characterized as an apoA-I receptor, triggering HDL internalization. This study was undertaken to identify the overexpression of ectopic ATP synthase β-chain on DIL-HDL uptake in primary hepatocytes in vitro and on plasma HDL levels in SR-BI knockout mice. Human ATP synthase β-chain cDNA was delivered to the mouse liver by adenovirus and GFP adenovirus as control. The adenovirus-mediated overexpression of β-chain was identified at both mRNA and protein levels on mice liver and validated by its increasing of DiL-HDL uptake in primary hepatocytes. In response to hepatic overexpression of β-chain, plasma HDL-C levels and cholesterol were reduced in SR-BI knockout mice, compared with the control. The present data suggest that ATP synthase β-chain can serve as the endocytic receptor of HDL, and its overexpression can reduce plasma HDL-C.

1. Introduction

Both epidemiological and clinical studies have demonstrated that the serum levels of high-density lipoprotein (HDL) cholesterol are inversely correlated with the risk of atherosclerosis [1–3]. HDL protects against atherosclerosis and cardiovascular disease by mediating reverse cholesterol transport, protecting vascular endothelium, and exerting antioxidant, anti-inflammatory, and antithrombotic effects [4, 5].

In humans, a 1 mg/dL (0.03 mmol/L) increase in baseline HDL is associated with a 6% decrease in the risk of death from coronary disease [6]. Furthermore, clinical trials have shown that HDL could be an important therapeutic target [7]. Therapy with the HDL mimetic apoAI phospholipid may result in regression of atherosclerosis [7], and these mimetic peptides may also influence the vascular biology of the vessel wall and protect against other acute and chronic inflammatory diseases [8]. Functional integrity of HDL is equally important for its antiatherogenic properties, as one study showed that patients with normal or elevated but functionally abnormal HDL suffered from atherosclerosis [9].

HDL takes up and transports surplus cholesterol from the peripheral tissues to the liver for disposal in bile [10], a process mediated by the HDL cell surface receptors on hepatocytes. Two receptor types have been identified, one is a high affinity receptor—scavenger receptor class B type I (SR-BI) [11] and the other is an endoreceptor—ATP synthase β-chain (ATPase-B1). SR-BI binds to HDL with a high affinity and may mediate selective cholesterol uptake of HDL in hepatocytes. SR-BI overexpression may significantly change plasma HDL levels, possibly reducing the incidence of arteriosclerosis [12–16]. Furthermore, SR-BI-knockout models show an increased rate of arteriosclerosis [17–19]. SR-BI is relatively nonspecific, as it binds to LDL,

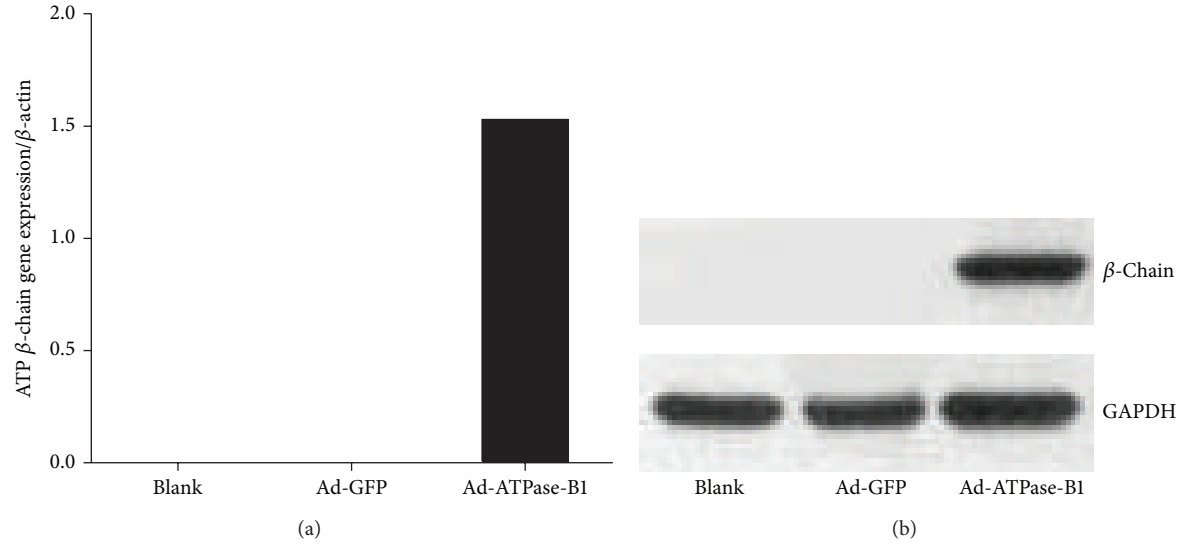

Figure 1: Amplification and identification of Ad-ATPase-B1 in HEK293A cells. Ad-GFP: adenovirus GFP; Ad-ATPase-B1: recombinant adenovirus ATP synthase β-chain. (a) Exogenous ATPase-B1 mRNA level in HEK293A cells with no vector (BLANK), pretreated with Ad-GFP and pretreated with Ad-ATPase-B1 and (b) ATPase-B1 protein detected with anti-V5-tag antibody (1 : 2000), GAPDH (1 : 1000).

oxidized LDL, and very low density lipoprotein in addition to HDL [20]. ATPase-B1 is also involved in HDL catabolism, as it triggers HDL and apoAI internalization in hepatocytes [21].

ATP synthase is an enzymatic complex (about 600 kDa) responsible for ATP synthesis in mitochondria, prokaryote membranes, and chloroplasts. Mitochondrial ATP synthase is composed of two domains: an extramembranous catalytic domain (F1) and a transmembrane domain (F0) that functions as a proton channel [22]. The mammalian ATP synthase consists of at least 16 different subunits: F1: α, β, γ, δ, and ε+IF1; F0: a–g, F6, A6L, and andoligomycin-sensitivity conferring protein [23]. It has also been found on the cell surface of endothelial cells, adipocytes, hepatocytes, and tumor cells using immunofluorescence or cell surface biotinylation techniques [24–28] and may be involved in neovascularization, hypertension, cell proliferation, and cytotoxicity [24–30], although the mechanism leading to its ectopic expression is still unknown.

The F1 domain of ATPase contains the catalytic site for ATP synthesis and hydrolysis and the binding sites for ATP and ADP [23]. In endothelial cells, apoA-I binding to ATPase-B1 causes ATP synthase to hydrolyze ATP into ADP and inorganic phosphate. ADP then stimulates apoA-I and HDL uptake into the cell and transendothelial transport of initially lipid-free apoA-I and HDL via activation of the P2Y12 receptor [31].

In the present study, we investigated whether overexpression of ATPase-B1 increases DiI-HDL uptake in primary hepatocytes of wild-type (WT) or SR-BI-knockout (SR-BI−/−) mice. We also sought to determine the effect of exogenously added ATPase-B1 cDNA on plasma HDL-C in SR-BI−/− mice.

2. Results

2.1. Construction and Amplification of Ad-ATPase-B1. To further determine the role of ATPase-B1 in HDL metabolism, we transferred human ATPase-B1 cDNA into an adenovirus vector, with a V5-tag on the carboxy terminal end for detection. Increased expression of ATPase-B1mRNA (Figure 1(a)) and protein (Figure 1(b)) was confirmed in HEK293A cells infected with Ad-ATPase-B1 compared with the control (Ad-GFP) vector.

2.2. ATPase-B1 Expression in Primary Hepatocytes. To study whether ATPase-B1 affects HDL uptake, we first confirmed its overexpression in primary hepatocytes. Primary hepatocytes infected with Ad-ATPase-B1 showed significantly higher levels of immunodetectable ATPase-B1 expression compared to the control vector (Figure 2).

To further investigate how different quantities of ATPase-B1 affect its cellular expression, we infected HepG2 cells with various concentrations of Ad-ATPase-B1 and Ad-GFP. Increased MOI corresponded to increased ATPase-B1 protein (Figure 3(a)) and mRNA expression (data not shown).

ATPase-B1 can be expressed on the surface of endothelial cells, adipocytes, hepatocytes, and tumor cells [24–28]. In order to determine MOI yielding the highest expression of Ad-ATPase-B1, we infected primary hepatocytes (WT and SR-BI−/− mice) with a range of different MOIs. While mRNA expression of Ad-ATPase-B1 was highest at 100 MOIs in the WT mice (Figure 3(b)), ATPase-B1 mRNA expression was highest at 30 MOIs in SR-B1−/− mice. Therefore, we used 30 MOIs Ad-ATPase-B1 for the remainder of our study. Unlike the HepG2 cells (Figure 3(a)), WT and SR-BI−/− hepatocytes showed slightly higher protein expression in response to higher MOIs (Figure 3(c))

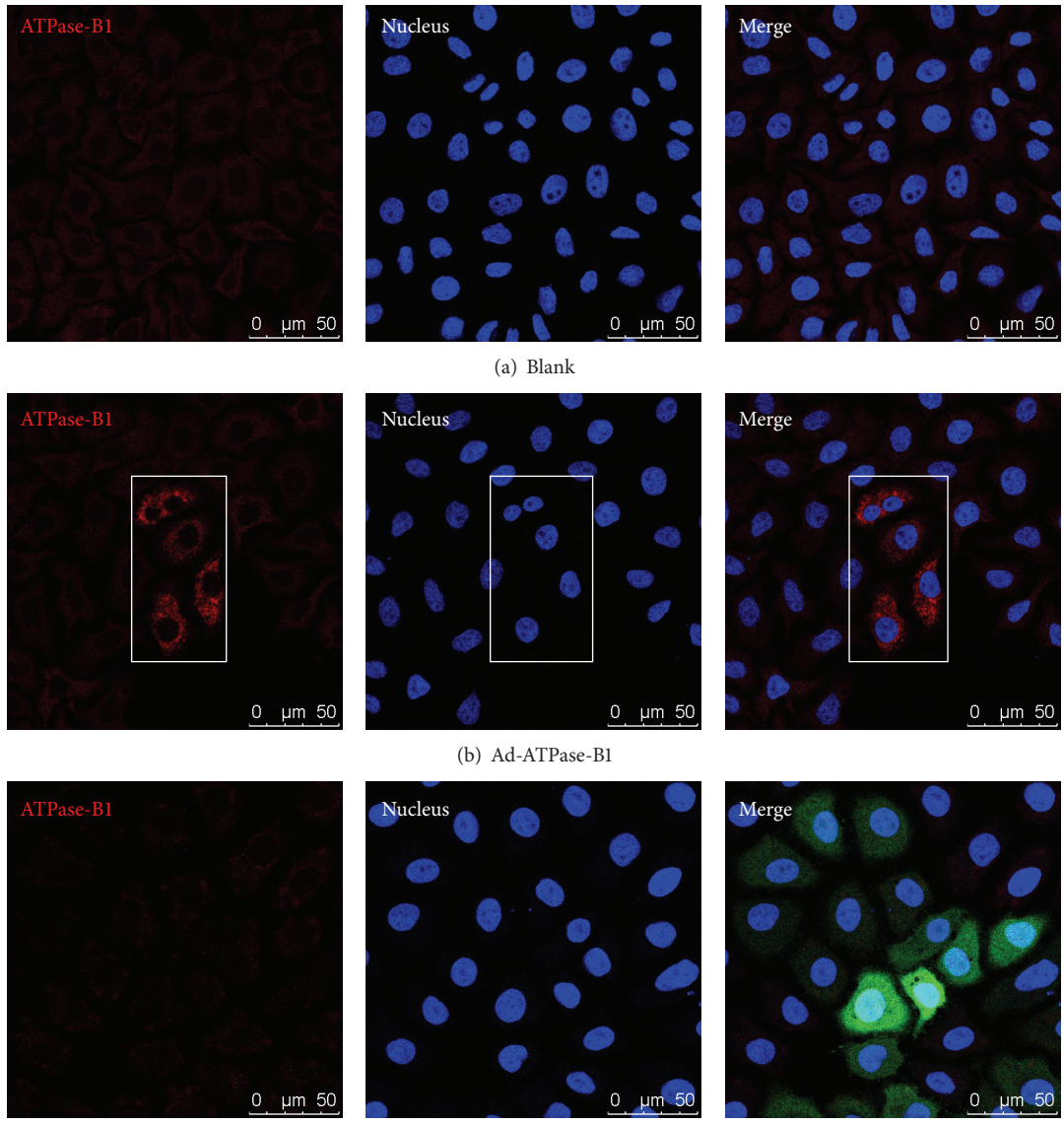

Figure 2: Immunofluorescent confocal microscopic analysis of ATPase-B1 expression in primary hepatocytes. Freshly isolated primary hepatocytes grown on cover slips were pretreated with Ad-ATPase-B1 (b) and Ad-GFP (c) at 30 MOIs for 48 h. After washing with PBS, the cells were immunostained with antibody against V5-tag and AlexaFluor 596-conjugated goat anti-mouse IgG and analyzed by confocal microscope.

2.3. ATPase-B1 Overexpression Increases DiI-HDL Uptake. We next investigated the effect of Ad-ATPase-B1 overexpression on DiI-HDL uptake. As shown in Figure 4(a), apoA-I (1 mg/mL) as the standard, the concentration of isolated HDL was 1 mg/mL, and DiI-HDL was 0.25 mg/mL. Freshly isolated primary hepatocytes were examined by fluorescence microscopy.

Figure 4(b) showed DiI-HDL uptake was significantly higher in Ad-ATPase-B1 infected WT and SR-BI−/− hepatocytes compared to their respective controls ($P < 0.01$). Although the DiI-HDL uptake is significantly higher in infected WT hepatocytes compared to the SR-BI−/− counterparts ($P < 0.01$), Ad-ATPase-B1 infection increased DiI-HDL uptake similarly in both groups (Table 1), suggesting that the lack of SR-BI does not affect ATPase-B1 function.

2.4. ATPase-B1 Overexpression Decreases Plasma HDL-C. Ectopic ATPase-B1 has been characterized as an apoA-I receptor, triggering HDL internalization in hepatocytes [21]. To further assess the role of liver ATPase-B1 in HDL metabolism, we injected WT and SR-BI−/− (1×10^9 pfu) mice with either Ad-ATPase-B1 or Ad-GFP via tail vein. Liver ATPase-B1 mRNA level increased significantly in both groups (Figure 5(a)); however, mRNA expression was significantly higher in the transfected SR-BI−/− mice ($P < 0.001$)

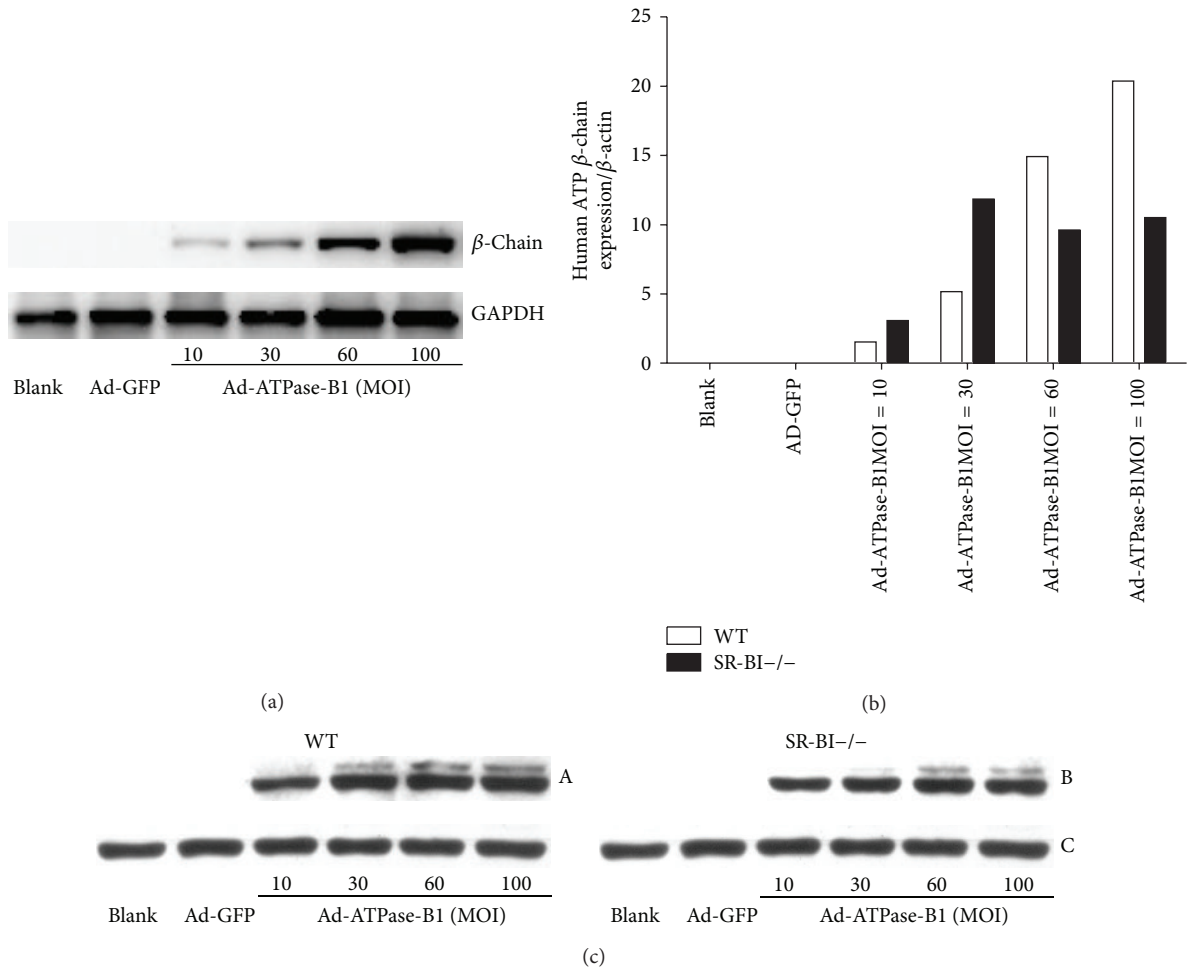

Figure 3: Multiplicity of infection (MOI) test in HepG2 cells and primary hepatocytes. HepG2 cells (a) and the isolated primary hepatocytes were pretreated with Ad-ATPase-B1 and Ad-GFP with different MOIs, and ATPase-B1 mRNA (b) and protein ((c) A, ATPase-B1 expression in WT primary hepatocytes; B, ATPase-B1 expression in SR-BI−/− primary hepatocytes; C, the internal control of GADPH in WT and SR-BI−/− primary hepatocytes) levels were measured. In HepG2 cells and WT hepatocytes, mRNA expression increased with increased MOI, while mRNA expression peaked at 30 MOIs in SR-BI−/− mouse.

compared to WT mouse. ATPase-B1 protein was detected in the livers of both WT and SR-BI−/− mice (Figure 5(b)).

Plasma total cholesterol and HDL-C were 2.5 and 3 times higher, respectively, in SR-BI−/− mice before infection (Figures 6(a)–6(c)). SR-BI−/− mice infected with Ad-ATPase-B1 showed lower plasma total cholesterol (~18%) and HDL-C (~12%) compared to SR-BI−/− mice infected with the control adenovirus (Figures 7(a)-7(b)), but plasma triglycerides were not different between the groups (Figure 7(c)). ATPase-B1 infection did not affect plasma total cholesterol, HDL-C, or triglycerides in WT mice (Figures 7(a)–7(c)). Analysis of lipoprotein profiles in the pooled plasma sample revealed lower HDL-cholesterol (Figure 8) in the Ad-ATPase-B1 mice compared to Ad-GFP mice. However, Ad-ATPase-B1 treated mice showed a significant increase in VLDL/CM-associated TG compared to Ad-GFP mice (Figure 8(a)).

3. Discussion

In this study, we set out to determine whether overexpression of ATPase-B1 affects plasma lipoprotein levels and whether this effect is mediated by SR-BI. ATPase-B1 is an enzyme located in the inner mitochondria membrane. A previous study showed that the surface β-chain is an apoA-I/HDL receptor [21], and the complex has been found on the cell surface of endothelial cells, adipocytes, hepatocytes, and tumor cells by immunofluorescence or after biotinylation of the cell surface [24–28]. Recently, new research has shown that knock-down of ABCA1, ABCG1, and SR-BI diminishes, but does not completely block, the transport of apoA-I or HDL through the endothelium [32, 33]. The ectopic presence of ATPase-B1 on the surface of endothelial cells was confirmed by cell surface biotinylation [31]. To investigate the effect of ATPase-B1 on HDL metabolism, we constructed an adenovirus containing the whole length of human ATPase-B1 and successfully transfected this Ad-ATPase-B1 into HEK293A cells (Figures 1(a) and 1(b)) and HepG2 cells (Figure 2(a)).

To further validate the role of ATPase-B1 on HDL metabolism, we measured DiI-HDL uptake after infecting primary hepatocytes with Ad-ATPase-B1. We demonstrate

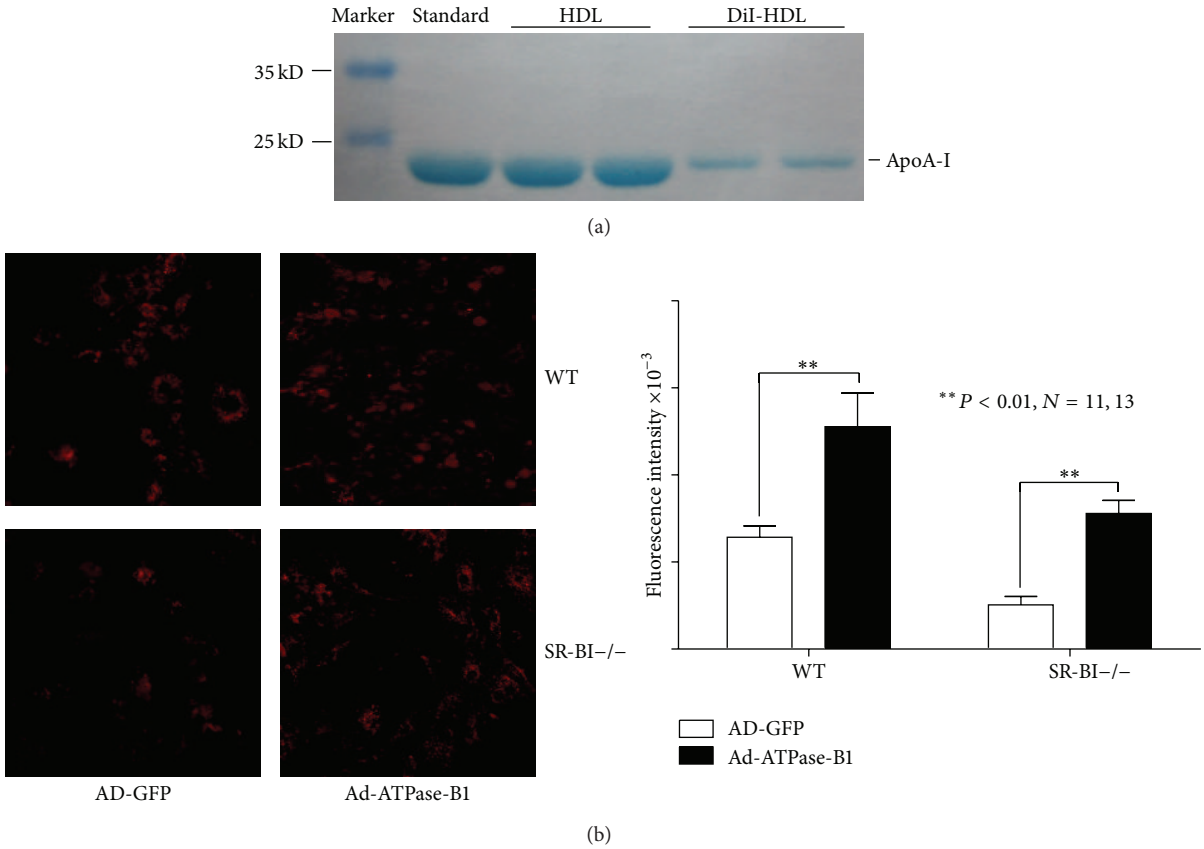

Figure 4: Analysis of DiI-labeled HDL uptake in freshly isolated primary hepatocytes (200x). Primary hepatocytes were pretreated with Ad-ATPase-B1 and Ad-GFP at 30 MOI for 48 h and incubated with 25 μg DiI-HDL for 2 h. (a) Concentration of DiI-HDL and HDL was determined by the dying method with Coomassie brilliant blue. (b) DiI-HDL was detected by fluorescence microscopy (200x) and cellular fluorescent intensities were quantified.

Table 1: Mean fluorescence intensity in WT and SR-BI−/− primary hepatocytes.

Type/virus	Ad-GFP	Ad-B1
WT	3.5	6.8**
SR-BI−/−	0.8	3.9**

**Ad-B1 compared with Ad-GFP groups $P < 0.01$. The increment was 3.3 in WT group, while it was 3.1 in SR-BI−/− group; the difference had no statistical significance.

that overexpression of hepatic ATPase-B1 by adenovirus infection increases DiI-HDL uptake (Figure 4(b)) in cultured primary hepatocytes and decreases plasma total cholesterol and HDL-C (Figure 7) in SR-BI−/− mice. These findings agree with previous studies demonstrating that significant amounts of immunodetectable β-chain protein were present on the HepG2 cell surface and increased HDL uptake [29].

Although the infected WT hepatocytes had a greater absolute DiI-HDL uptake compared to the SR-BI−/− mice ($P < 0.01$), the difference in uptake between ATPase-B1 and GFP-infected mice was similar in both mouse models, suggesting that ATPase-B1 does not interact with SR-BI in increasing DiI-HDL uptake. Previous research in SR-BI−/− mice showed that plasma total cholesterol, HDL, and HDL volume were double that of a normal mouse [34, 35]. ATPase-B1 overexpression also decreased plasma total cholesterol (~18%) and HDL-C (~12%) (Figures 7(a)-7(b)) and showed a depletion of HDL-C lipoprotein profiles (Figure 8). However, our ATPase-B1 adenovirus is not specifically located in the cell membrane. Therefore, our infection procedure may not fully reflect the function of ATPase-B1 in HDL metabolism *in vivo*. Constructing an adenovirus specifically located in the cell membrane will help us understand the role of ATPase-B1 in HDL metabolism more clearly.

The exact mechanism of ATPase-B1 on the cell surface remains unclear. Previous research in hepatocytes shows that the β-chain functions as an apoA-I receptor and triggers HDL endocytosis [21]. Upon binding of apoA-I, ATP synthase hydrolyzes ATP into ADP and inorganic phosphate, and ADP stimulates hepatic HDL uptake by activating the purinergic receptor P2Y13 through the small GTPase RhoA and its effector ROCK I [28, 36]. However, whether this pathway is altered in SR-BI−/− mice is still unknown. Then, we will go through this pathway in future work.

Many genes participate in HDL and apoA-I metabolism, such as ATP-binding cassette transporter A1 (ABCA1) [37], ATP-binding cassette transporter G1 (ABCG1) [38–40], and

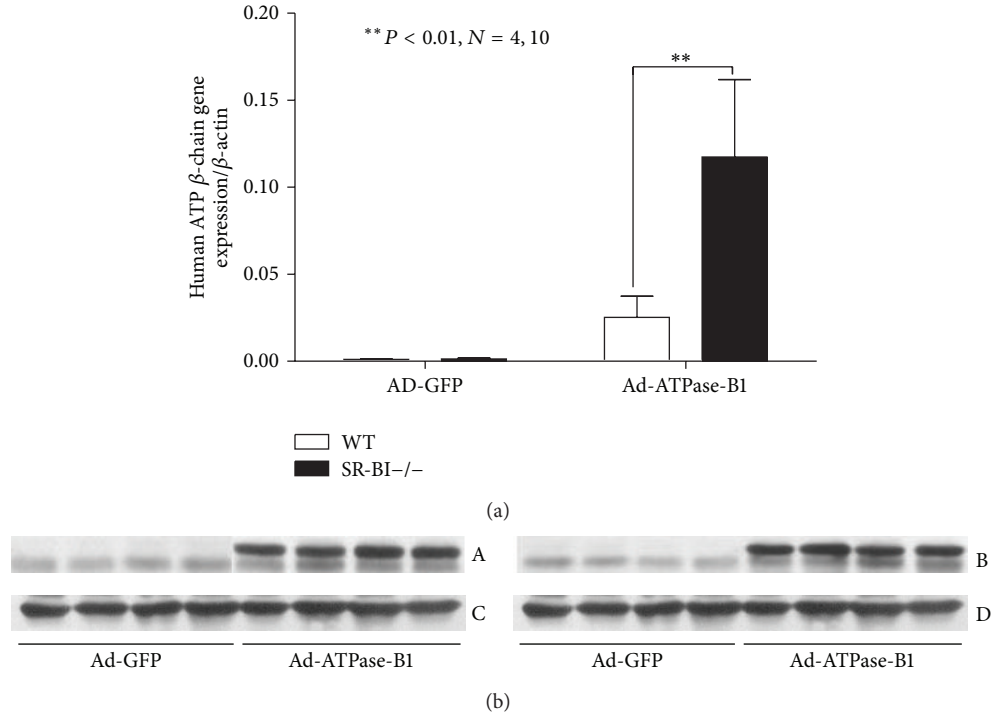

Figure 5: Verification of ATP-B1 expression in the livers of WT and SR-BI−/− mice by RT-PCR (a) and Western blotting ((b) A, ATPase-B1 expression in WT mouse liver; B, ATPase-B1 expression in SR-BI−/− mouse livers; C and D, the internal control of GADPH) after 7 days injection of Ad-ATPase-B1 and Ad-GFP. ($^{**}P < 0.001$; $n = 4, 10$).

SR-BI. However, whether ATPase-B1 interacts with them in HDL endocytosis is still unclear. Coinhibition experiments in endothelial cells suggest that ABCA1, ABCG1, SR-BI, and ATPase-B1 interact in a series of events rather than on independent parallel processes [31]. Future studies should aim to measure plasma apoA-I levels and determine whether there is an interaction among ATPase-B1, ABCA1, ABCG1, SR-BI, and apoA-I and the potentially downstream signaling pathway involved.

In summary, the present study demonstrated that overexpression of ATPase-B1 increased DiI-HDL uptake in primary hepatocytes and reduced plasma HDL-C and total cholesterol in SR-BI−/− mice and that ATPase-B1 increased HDL uptake independently of the presence of SR-BI. Future research should investigate the effect of ATPase-B1 on plasma apoAI and characterize the signaling events and downstream targets for HDL endocytosis.

4. Materials and Methods

4.1. Cell Culture. HepG2 and HEK293A cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in DMEM containing 10% FBS (GIBCO, California, USA) supplemented with 100 U/mL penicillin G and 100 μg/mL streptomycin sulfate at 37°C in a humidified atmosphere of 95% air, 5% CO_2. For adenovirus infection experiments, cells were seeded in six-well plates at a density of 2×10^5 cells per well and incubated in DMEM containing 5% FBS. Cells were infected with either Ad-ATPase-B1 or the control vector Ad-GFP.

4.2. Animal Studies. SR-BI−/− mice and WT mice were fed standard rodent chow and water *ad libitum* in sterile cages with a 12 h light/dark cycle. All mice used in this study were 12-week-old females. All the procedures involving mice were approved by Animal Ethics Committee. Two hundred microliters of a 0.9% sterile solution containing 1×10^9 pfu of either Ad-ATPase-B1 or control Ad-GFP adenovirus was injected into the tail vein. Mice were fasted for 4 h and 200 μL aliquots of orbital venous blood were collected after anesthesia into heparin-coated capillary tubes at 0 and 7 days. Plasma was collected by centrifuging samples at 4000 rpm for 10 min at 4°C and subsequently stored at −80°C. After 7 days, mice were anesthetized by intraperitoneal injection of pentobarbital, and tissue and blood were collected. Plasma levels of TG and cholesterol were determined using Thermo Infinity TG and cholesterol reagents (ThermoElectron, Melbourne, Australia). Plasma HDL-C levels were extracted using PEG precipitation and then determined using Thermo Infinity cholesterol reagents.

4.3. Recombinant Adenoviruses. ATPase-B1 cDNA was cloned into the plasmid pAd. The plasmid was linked to CMV using the restriction enzymes Bgβ and Sph, with a V5-tag added to the C-terminal for detection purposes. CMV-β-chain was linearized with Nhe and cotransfected into HEK293A cells with Ad-DNA. The recombinant virus

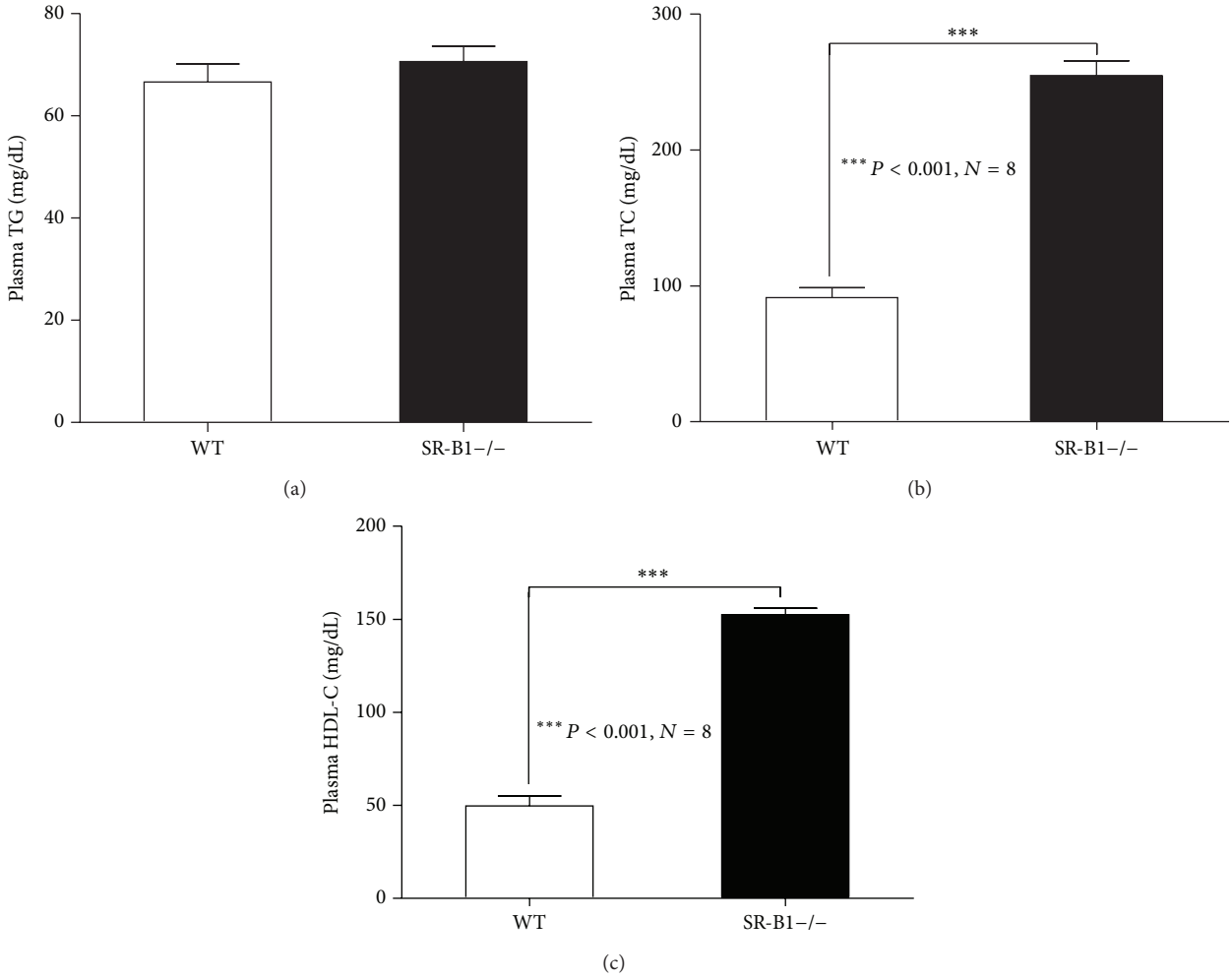

Figure 6: Plasma lipid levels in WT and SR-BI−/− mice. Before injection, plasma was collected after a 4 h fast. Plasma triglyceride (a), total cholesterol (b), and HDL-C (c) levels were detected.

Ad-ATPase-B1 was plaque-purified, expanded, and purified on CsCl gradients as described by Kozarsky et al. [41]. The control adenovirus Ad-GFP was constructed using the same procedure but without the transgene expression cassette.

4.4. Primary Hepatocyte Isolation and Culture. Hepatocytes were isolated from WT and SR-BI−/− mice. The portal vein was cannulated, and the liver was perfused with KRG buffer (pH 7.4) containing 120 mM NaCl, 480 mM KCl, 120 mM $MgSO_4$, 120 mM KH_2PO_4, and 50 mM EGTA. After perfusing at a rate of 1 mL/min at 37°C for 20 min, 50 mL collagenase buffer containing 1 mM CaCl and IV collagenase was added and perfusion continued for an additional 20 min. Cells were filtered to remove undigested fragments, centrifuged for 4 sec at 50 g, and washed twice in cold culture medium to remove damaged and nonliver cells. Isolated cells were seeded on six-well plates at a density of 3×10^5 cells per well in DMEM medium containing 10% FBS. Culture medium was changed 4 h after seeding. The plated cells were infected with Ad-ATPase-B1 (titer: 8×10^{10} pfu/mL) in 1 mL of fresh DMEM at 37°C for 36 h at 30 multiplicities of infection (MOIs). One milliliter of complete medium was then added to each well. Parallel experiments were conducted using the control adenovirus vector (Ad-GFP, titer: 1×10^{11} pfu/mL) to infect the cells at the same MOI dose to determine the effect of adenovirus alone on the cells.

4.5. HDL Isolation and Labeling. Human HDL ($1.063 < d < 1.21$) was isolated from serum using density gradient centrifugation according to Redgrave et al. [42]. To remove KBr buffer, the isolated HDL was dialyzed with PBS containing EGTA-Na_2 and stirred at 4°C for 48 h. The HDL was labeled with the fluorescence probe DiI (Beyotime) according to Pitas et al. [43] and was also dialyzed. HDL and DiI-HDL showed no differences in apoprotein composition on 12% SDS-PAGE [44].

4.6. HDL Uptake Assay. Primary hepatocyte uptake of DiI-HDL was analyzed using a fluorescence microscope. Briefly, the infected cells were cultured for 48 h and then incubated with 25 μg DiI-HDL (0.25 mg/mL) at 37°C for 2 h in DMEM medium containing 5% FBS. Cells were then washed three times with PBS and fixed with 4% paraformaldehyde for

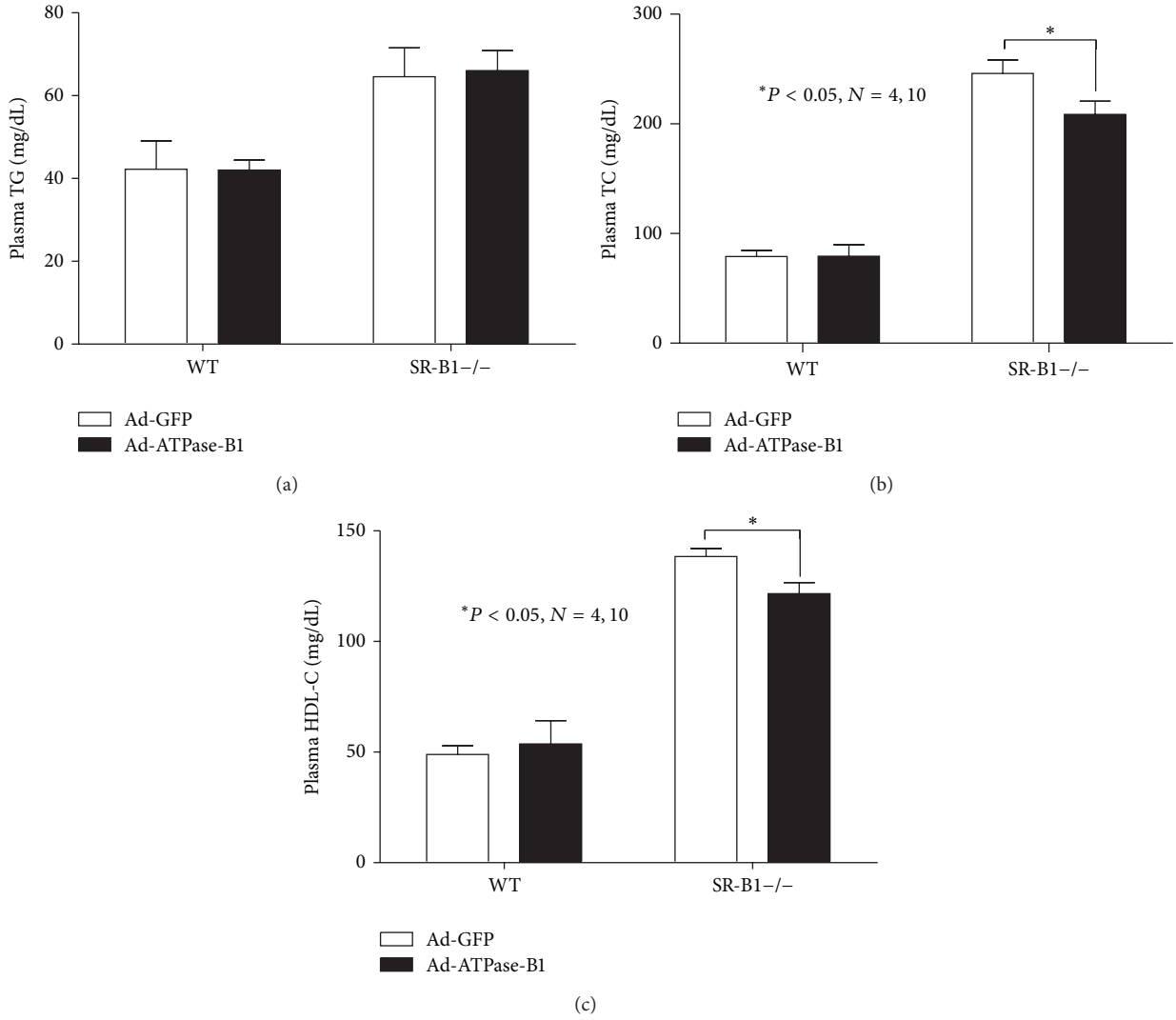

Figure 7: Plasma triglyceride (a), total cholesterol (b), and HDL-C (c) levels in WT and SR-BI−/− mice 7 days after administration of Ad-ATPase-B1 and Ad-GFP.

20 min at room temperature. Stained cells were imaged with fluorescence microscopy.

4.7. Western Blot Analysis. All cell and liver proteins were extracted following the standard protocol [45]. Proteins were separated on 10% SDS-PAGE gels and transferred onto nitrocellulose membranes. ATPase-B1 expression was analyzed using a mouse-anti-V5 monoclonal antibody (1 : 2000), primary antibody, and a goat-anti-mouse IgG-HRP (1 : 5000) secondary antibody. GAPDH (1 : 1000 dilution for the primary antibody and 1 : 5000 dilution for the secondary antibody) was used as an internal control.

4.8. RT and Quantitative Real-Time PCR. Total RNA extracted from cells and livers was reverse-transcribed using 10 units of M-MLV reverse transcriptase (Promega) following the standard procedure [46]. Real-time PCR was performed using QuantiTect SYBR Green PCR reagents (Life Technologies, California, CA). ATPase-B1 (TGGTGGTGCTGGAGTTGG, GCCTGGGTGAAGCGAAAG) transcription levels were normalized to β-actin (CGTGGGCCGCCCTAGGCACCA, TTGGCCTTAGGGTTCAGGGGGG).

4.9. Fast-Protein Liquid Chromatography Fractionation of Lipoproteins. Plasma aliquots (250 μL) were pooled from a group of mice and applied to Tricorn high-performance Superose S-6 10/300 GL columns using a fast-protein liquid chromatography system (Amersham Biosciences), followed by elution with PBS at a constant flow rate of 0.25 mL/min. Eluted fractions (500 μL) were assayed for TG and cholesterol concentrations using the TG and cholesterol kits (BioSino, China).

4.10. Confocal Microscopy. Freshly isolated primary hepatocytes grown on cover slips were pretreated with Ad-ATPase-B1 (A) and Ad-GFP (B) (MOI = 30) for 48 h. After three

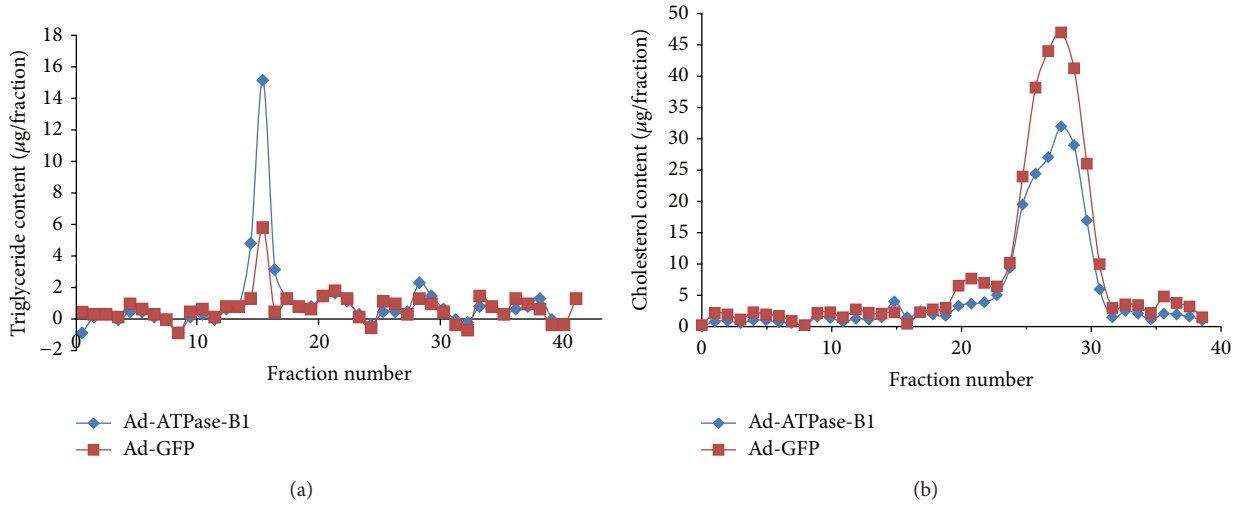

Figure 8: Plasma lipoprotein profiles. Seven days after Ad-ATPase-B1 and Ad-GFP administration, mice were fasted for 4 h and euthanized. Aliquots (250 μL) of plasma pooled from each group of mice were fractionated by column gel filtration chromatography. Fractions (500 μL) were eluted and assayed for TG (a) and cholesterol (b) levels.

PBS washes, cells were fixed with 4% paraformaldehyde at room temperature for 5 min. After washing with PBS, the cells were immunostained with an antibody against V5-tag (Life Technologies, California, CA) (5 mg/mL) in PBS-1% BSA and AlexaFluor 596-conjugated goat anti-mouse IgG (5 mg/mL) (Molecular Probes) and analyzed using confocal microscopy (Leica SP5, Germany).

4.11. Statistics. Statistical analyses were performed using the PRISM statistics software. Analysis of variance (ANOVA) was used to compare the data. All experiments were performed in triplicate, and representative results are presented. Quantitative data are expressed as mean ± S.D. Student's t-test was used for statistical comparisons. $P < 0.05$ was considered statistically significant.

Abbreviations

ATPase-B1: Adenovirus containing human holo-length ATP synthase β-chain
DiI-HDL: HDL was labeled with fluorescent dye (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate)
HDL: High-density lipoprotein
SR-BI: Scavenger receptor class B, type I.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Kexiu Song, Yingchun Han, Linghua Zhang, and Guoqing Liu are the major contributors who executed the study and performed data analysis. Peng Yang and Xiaoyun Cheng conducted animal studies and performed blood chemistries. Le Bu performed studies in cultured hepatocytes. Hui Sheng performed confocal microscopy. Shen Qu designed the study, performed statistical analysis of data, and wrote the paper. Kexiu Song and Yingchun Han contributed equally to this work and are the co-first authors.

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