diabetology

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Surrogate Indexes of Insulin Resistance Are Affected by Sex and Gender and by the Combination of Smoking and Oral Contraceptives

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Abstract: Background: Surrogate indexes of insulin resistance (IR) are less expensive than the euglycemic glucose clamp. The simultaneous impact of sex and gender, smoking, and combined oral contraceptives (COC) on IR surrogate indexes was studied in a cohort of healthy young men and women (stratified in COC-free women and COC users). Methods: Glycemia, insulin, C-peptide, TG, and HDL were measured in serum samples and used to calculate IGR, HOMA-IR-IR, QUICKI, FIRI, METS-IR, TG, and MCAi. Results: Men had higher BMI, glycemia, TG, METS-IR, TyG, and lower HDL than COC-free women and they had lower IGR and higher METS-IR and MCAi than COC users. TG, HDL, and TyG were lower and MCAi is higher in COC-free women than in COC users. In non-smokers, men had higher BMI and METS-IR and lower HDL than both cohorts of women. COC-free women showed a lower TyG index than men and COC women and lower TG, HDL, and IGR. MCAi was higher in COC-free women than in COC users. Smoking reduced sex and gender differences: HDL was lower in men than COC users and IGR was lower in men than COC-free women. Intra-sex differences were reported only in COC-free women: smokers had higher insulin, C-peptide, TG, and IGR and lower MCAi than non-smokers. Cluster analysis evidenced a significant separation between the sexes and smokers and non-smokers. Conclusions: Smoking leads to changes in the phenotype of both men and women, as well as COC in women; they should be considered independent variables in clinical studies given, representing a fundamental cornerstone in the personalization of prevention and care.

Keywords: tobacco smoking; combined oral contraceptives; sex and gender differences; insulin resistance; insulin resistance indexes; humans

Citation: Tonolo, G.; Montella, A.; Puci, M.V.; Sotgiu, G.; Muresu, N.; Cherchi, S.; Palermo, M.; Seghieri, G.; Franconi, F.; Campesi, I. Surrogate Indexes of Insulin Resistance Are Affected by Sex and Gender and by the Combination of Smoking and Oral Contraceptives. *Diabetology* **2024**, *5*, 677–689. https://doi.org/10.3390/diabetology5070050

Academic Editor: Tessa Lee Crume

Received: 25 September 2024
Revised: 20 November 2024
Accepted: 27 November 2024
Published: 5 December 2024

1. Introduction

Over the past few decades, sex and gender differences have gained a high priority in prevention and care [1,2]. Recent guidelines strongly suggest that sex and gender differences should be considered in preclinical and clinical research and clinical settings to avoid the traditional male predominance, which leads to inappropriateness in women prevention and care [2–4], including metabolic disorders such as glucose homeostasis [3]. Insulin sensitivity is higher in adult women [3] and they are more protected from endothelial

insulin resistance (IR), a precursor of IR induced by obesogenic diets [5]. In addition, there is a female-specific risk of IR such as gestational diabetes. Premenopausal women have a lower IR than men, while postmenopausal women are more prone to develop IR than premenopausal women; interestingly, "women protection" can be restored by hormonal replacement therapy [5,6], indicating that sexual hormones can play a role in IR.

IR is a complex condition characterized by beta cell dysfunction, excessive insulin secretion, and impaired glucose tolerance and is associated with the so-called Western lifestyle [7]. IR is linked with chronic low-grade inflammation and is a risk factor for diabetes mellitus type 2, cardiovascular diseases (CVD), chronic kidney disease, Alzheimer's disease, and cancer [7], and its early diagnosis could reduce the risk of several chronic diseases.

The gold standard for IR diagnosis is the euglycemic glucose clamp technique, which is expensive, time-consuming, and not easily accessible [8]. Therefore, surrogate indexes such as the insulin/glucose ratio (IGR), homeostasis model assessment (HOMA-IR) (glycemia mmol/L $\times$ insulin μU/mL)/22.5), quantitative insulin sensitivity check index (QUICKI)(1/(ln(insulin μU/mL) + ln(glycemia mg/dL), fasting insulin resistance index (FIRI) (glycemia mmol/L x insulin μU/mL/25), the metabolic score for insulin resistance (METS-IR) (Ln((2 $\times$ glycemia mg/dL) + triglycerides (mg/dL)$\times$BMI)/(Ln(HDL mg/dL), triglyceride-glucose index (TyG) (ln [triglycerides mg/dL $\times$ glycemia mg/dL]/2), and Mcauley index (MCAi: exp [2.63$-$0.28$\times$ ln(insulin μU/mL) $-$0.31 $\times$ Ln triglycerides mg/dL) were developed [8]. In particular, IGR, HOMA-IR, FIRI, TyG, and METS-IR measure IR, whereas QUICKI and MCAi measure insulin sensitivity [9,10].

IR risk factors are numerous and include smoking, sex, and gender. Smoking, directly and indirectly, induces IR in a dose-dependent way [11] that is affected by sex and gender [12,13]. In real life, smoking can modify male and female phenotypes although the consequences of this variation probably occur differently in men and women [14,15]. As already mentioned, IR in women is affected by exogenous and endogenous sex hormones [5,6], and numerous interactions are described between combined oral contraceptives (COC) and smoking [14,15], including a major thrombotic risk [16]. Therefore, the present study aimed to investigate the simultaneous impact of sex, gender, and smoking on surrogate indexes of IR in a cohort of healthy young men and women. Furthermore, the female cohort is further stratified in COC-free women and COC users to analyze the impact on IR of the combination of COC and smoking.

2. Materials and Methods

2.1. Study Design and Participants

The study was approved by the Ethical Committee (prot. PG/2019/6280). All procedures were conducted following the Helsinki Declaration.

During a voluntary blood donation, this observational study consecutively enrolled men and women (women have regular menstrual cycles of 28 days) and women taking COC. Individuals (aged between 18 and 40 years) were healthy and non-obese (no kidney, liver, heart, endocrine, or infectious diseases in the previous 2 months) and not exposed to chronic therapies, with the only exception of COC. Sampling in women was carried out during the follicular phase of the menstrual cycle (1–10 days). The cohorts were stratified based on smoking habits, with smokers (22 men, 24 women, and 13 COC users) defined as subjects who smoked at least one cigarette per day during the enrollment period, as evidence indicates that even a few cigarettes per day for short periods are associated with IR [17]. The COCs used by the volunteers consisted of ethinyl estradiol (with a prevalence of 20 μg dose) and gestodene, drosperinone, desogestrel, levonorgestrel, and chlormadinone as progestins (with a prevalence of 3 mg dose).

2.2. Laboratory Examinations

Standard laboratory assays were used to assess glycemia (glucose oxidase technique on an automated analyzer), insulin (CLIA assay, LIAISON Insulin kit; DiaSorin, Saluggia (VC), Italy), C-peptide (CLIA assay, LIAISON C-peptide kit; DiaSorin, Saluggia (VC), Italy),

triglycerides (TG), and HDL in serum. These values were then used to derive the following insulin-related indexes: IGR (U/mol), HOMA-IR-IR, QUICKI, FIRI, METS-IR, TG, and MCAi.

2.3. Statistical Analysis

Data were described using the mean and standard deviation (SD) or median and interquartile range (IQR) and absolute and relative (percentage) frequency for quantitative and qualitative variables, respectively.

Spearman's correlation coefficients were calculated to assess the relationship between BMI and collected variables.

A hierarchical cluster analysis using Gower distance (mixed variables) was performed to identify homogeneous groups/clusters.

Differences in qualitative variables were evaluated by Pearson Chi or Fisher exact tests. Whereas, for quantitative variables (2 groups) unpaired Student *t* or Mann–Whitney tests were used. For other comparisons (3 groups) one-way ANOVA or Kruskal–Wallis tests were applied. Bonferroni correction for multiple testing was performed. A two-tailed *p*-value less than 0.05 was considered significant. Statistical analyses were carried out using STATA®17 (StataCorp, College Station, TX, USA).

3. Results

3.1. Cohorts Before Smoking Stratification

A total of 167 healthy subjects (55 men, 56 COC-free women, and 56 COC women) were enrolled. The mean age of the three non-obese cohorts (men, COC-free, and COC women) was similar (Table 1). Fasting glucose, fasting insulin, TG, HOMA-IR, QUICKI, FIRI, MCAi, and TyG were normal (Table 1) [18–21].

Men had higher body mass index (BMI), glycemia, TG, METS-IR, TyG, and lower HDL than COC-free women; when compared with COC-users, men had lower IGR and higher METS-IR and MCAi. COC-free women showed lower levels of TG, HDL, and TyG and higher MCAi in comparison with COC users (Table 1).

Table 1. The characteristics of the cohorts before stratification for smoking.

Variables	Men (*n* = 55)	COC-Free Women (*n*= 56)	COC Women (*n*= 56)	*p*-Value
Smoke, n (%)	22 (40.0)	24 (42.9)	13 (23.6)	0.08
Age (years)	27.5 (4.3)	27.6 (4.8)	27.5 (3.9)	0.97
BMI (kg/m^2)	23.5 (2.8) *	22.1 (4.4)	21.4 (2.3)	0.003
Glycemia (mg/dL)	81.4 (10.1) *	76.4 (11.4)	77.2 (8.4)	0.02
Insulin (µU/mL)	5.8 (3.9–8.4)	5.9 (4.2–8.3)	7.1 (5.4–8.6)	0.09
C-peptide (ng/mL)	1.6 (1.3–2.0)	1.6 (1.4–2.0)	1.7 (1.4–2.3)	0.63
TG (md/dL)	73 (58–103) *	67.5 (56–79.5) ^	89 (68–122)	0.0004
HDL (mg/dL)	50 (44–59) *	61.0 (51.5–66.0) ^	70 (62–79)	<0.0001
IGR (U/mol)	1.3 (0.9–1.72) °	1.3 (1.0–1.9)	1.7 (1.3–2.0)	0.02
HOMA-IR	1.2 (0.8–1.6)	1.1 (0.8–1.5)	1.4 (0.9–1.7)	0.22
QUICKI	0.38 (0.03)	0.38 (0.03)	0.37 (0.03)	0.14
FIRI	1.1 (0.7–1.4)	1.0 (0.7–1.3)	1.2 (0.8–1.5)	0.22
METS-IR	32.4 (5.6) *,°	27.1 (6.0)	26.8 (3.4)	<0.0001
TyG	4.38 (0.2) *	4.27 (0.2) ^	4.42 (0.2)	0.0007
MCAi	6.2 (0.6) °	6.3 (0.5) ^	5.9 (0.4)	0.0007

Data are expressed as mean (SD) or as medians (IQR). Post-hoc comparison: * statistically significant difference between men and COC-free women; ° statistically significant difference between men and COC women; ^ statistically significant difference between COC-free and COC women.

In men, BMI showed a positive correlation with age, insulin, C-peptide, TG, IGR, HOMA-IR, FIRI, TyG; conversely, a negative correlation for QUICKI and MCAi was observed (Table 2). In COC-free women, BMI did not correlate with any of the studied parameters. Whereas, in women who used COC, BMI was positively correlated with insulin, IGR, HOMA-IR, and FIRI,

while QUICKI was negatively correlated with BMI. These results indicated that COC users had more similarities with men than with COC-free women.

Table 2. Spearman correlation coefficient between BMI and collected variables in the three cohorts before smoking stratification.

BMI	Men (n = 55)	COC-Free Women (n = 56)	COC Women (n = 56)
Age	**0.36 (0.01)**	−0.10 (0.48)	−0.09 (0.52)
Glycemia (mg/dL)	0.23 (0.09)	−0.11 (0.41)	−0.02 (0.92)
Insulin (µU/mL)	**0.37 (0.01)**	0.07 (0.64)	**0.39 (0.003)**
C-peptide (ng/mL)	**0.30 (0.03)**	−0.08 (0.60)	0.24 (0.11)
TG (md/dL)	**0.28 (0.04)**	−0.10 (0.47)	−0.15 (0.26)
HDL (mg/dL)	−0.06 (0.67)	−0.05 (0.72)	−0.12 (0.38)
IGR (U/mol)	**0.33 (0.01)**	0.11 (0.41)	**0.37 (0.005)**
HOMA-IR	**0.40 (0.003)**	0.02 (0.89)	**0.33 (0.01)**
QUICKI	**−0.40 (0.003)**	0.01 (0.94)	**−0.33 (0.01)**
FIRI	**0.40 (0.003)**	0.02 (0.91)	**0.33 (0.01)**
TyG	**0.33 (0.01)**	−0.21 (0.12)	−0.17 (0.22)
MCAi	**−0.44 (0.001)**	0.02 (0.89)	−0.12 (0.37)

3.2. Smoking Stratification: Differences Between Non-Smokers and Smokers

Non-smoker men had higher BMI and METS-IR and lower HDL in comparison with both cohorts of non-smoker women (Table 3). COC-free women showed a lower TyG index than men and COC women. Moreover, significantly lower levels of TG, HDL, IGR, and higher MCAi were observed in COC-free women in comparison with COC users (Table 3).

Smoking stratification reduced sexually divergent parameters. However, HDL was significantly lower in smoking men than in COC users, whereas IGR was lower in men than in COC-free women (Table 3).

Table 3. Statistically significant sex differences following stratification by smoking.

Variables	Non-Smokers				Smokers			
	Men (n = 33)	COC Free Women (n = 32)	COC Women(n = 42)	p-Value	Men (n = 22)	COC Free Women (n = 24)	COC Women (n = 13)	p-Value
BMI (kg/m^2)	23.8 (2.4) *,°	22.4 (4.8)	21.1 (2.1)	**0.003**	23.2 (3.3)	21.7 (3.9)	22.2 (2.9)	0.36
TG (md/dL)	75 (58–103)	63.5 (50.5–72.5) ^	84 (70–122)	**0.0005**	72.5 (58–102)	73 (62.5–90)	95 (68–114)	0.31
HDL (mg/dL)	50.3 (11.6) *,°	60.9 (10.0) ^	71.4 (14.9)	**<0.0001**	51 (45–62) °	58.5 (51.0–66.5)	69 (61–75)	**0.02**
IGR (U/mol)	1.4 (1.1–1.9)	1.2 (0.9–1.8) ^	1.7 (1.3–2.1)	**0.02**	1.1 (0.9–1.5) *	1.6 (1.1–2.5)	1.6 (1.2–1.7)	**0.01**
METS-IR	32.8 (31.0–35.2) *,°	26.9 (25.4–32.9)	26.7 (25.1–29.7)	**0.0001**	31.1 (28.7–35.0)	27.7 (24.6–31.4)	30.2 (26.0–31.8)	0.10
TyG	4.4 (0.2) *	4.2 (0.2) ^	4.4 (0.2)	**0.0009**	4.4 (0.3)	4.3 (0.2)	4.4 (0.2)	0.25
MCAi	6.2 (0.5)	6.5 (0.5) ^	6.0 (0.4)	**0.0001**	6.3 (0.6)	6.1 (0.4)	6.0 (0.4)	0.15

Data are expressed as mean (SD) or as medians (IQR). Post-hoc comparison: * statistically significant difference between men and COC-free women; ° statistically significant difference between men and COC women; ^ statistically significant difference between COC-free and COC women.

Insulin-IGR, insulin-HOMA-IR, insulin-FIRI, TG-TyG, IGR-HOMA-IR, IGR-FIRI, and HOMA-IR-FIRI were positively related between them while insulin-QUICKI, IGR-QUICKI, HOMA-IR-QUICKI, and QUICKI-FIRI were positively related between them (Figures 1–3). Moreover, a positive correlation between QUICKI-MCAi and a negative one between insulin-MCAi, HOMA-IR-MCAi, and FIRI-MCAi were found except for COC women who smoke (Figures 1–3).

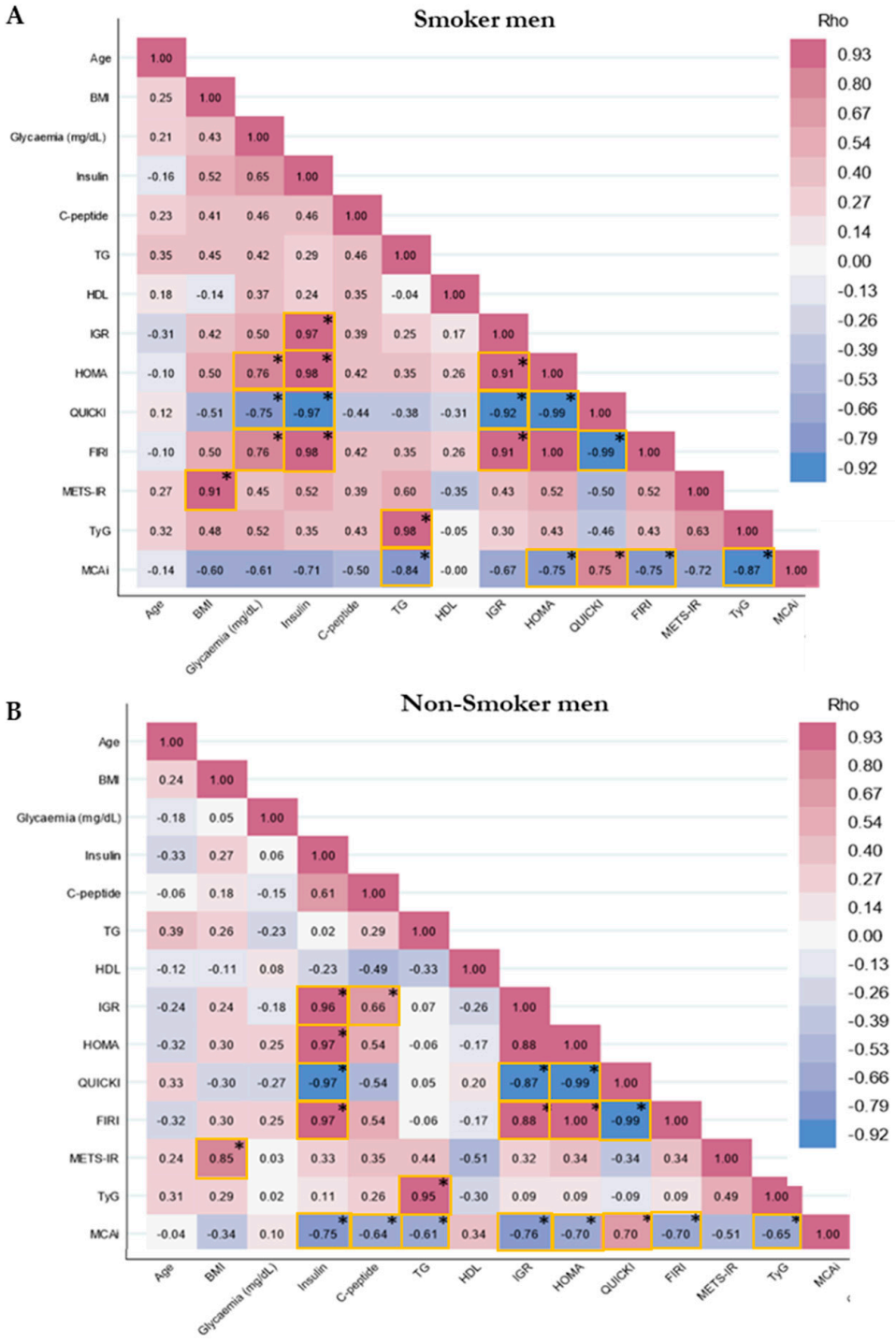

Figure 1. Heat maps of correlations in smoker (**A**) and non-smoker (**B**) men. Values represent Spearman's correlation coefficient. * in the yellow boxes represents the statistically significant correlations.

Furthermore, positive correlations were measured between glycemia-HOMA-IR and glycemia-FIRI and negative ones between glycemia-QUICKI and METS-IR-MCAi only in smoker men (Figure 1A). The relationship between insulin-C-peptide and C-peptide-IGR (positive), and C-peptide-MCAi and IGR-MCAi (negative) was detected only in non-smoker men (Figure 1B).

Finally, non-smoker COC-free women differed from smoker COC-free women in three negative correlations, TG-MCAi, IGR-MCAi, and TyG-MCAi, which were absent in smoker COC-free women (Figure 2A,B).

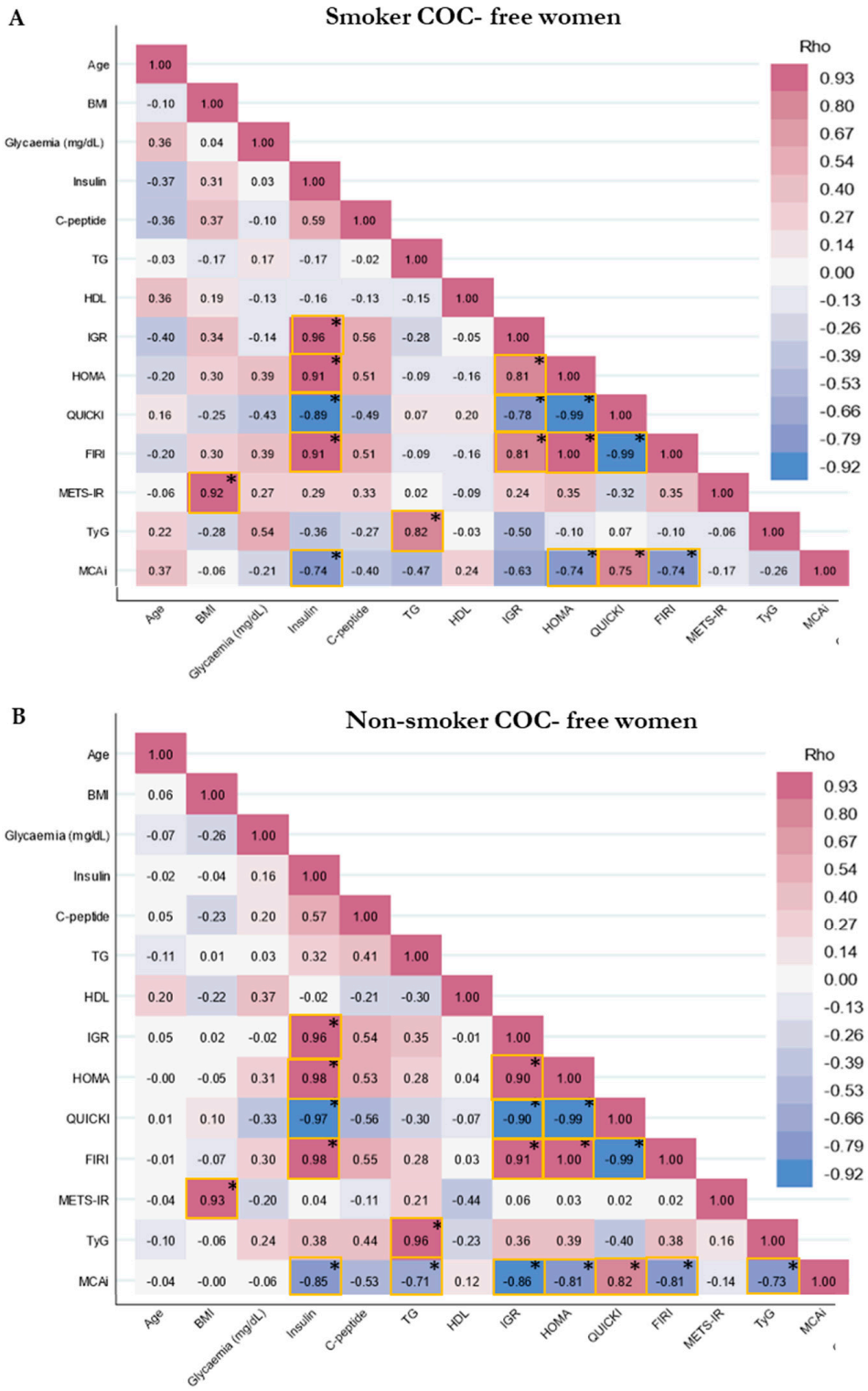

Figure 2. Heat maps of correlations in smoker (**A**) and non-smoker (**B**) COC-free women. Values represent Spearman's correlation coefficient. * in the yellow boxes represents the statistically significant correlations.

Interestingly, 14 more correlations were described only in non-smoker COC women (Figure 3B): 5 were positive correlations (insulin-C-peptide, C-peptide-IGR, C-peptide-HOMA-IR, C-peptide-FIRI, and QUICKI-MCAi) and 9 were negative correlations (glycemia-QUICKI, insulin-MCAi, C-peptide-QUICKI, TG-MCAi, HDL-METS-IR, IGR-MCAi, HOMA-IR-MCAi, FIRI-MCAi, and TyG-MCAi).

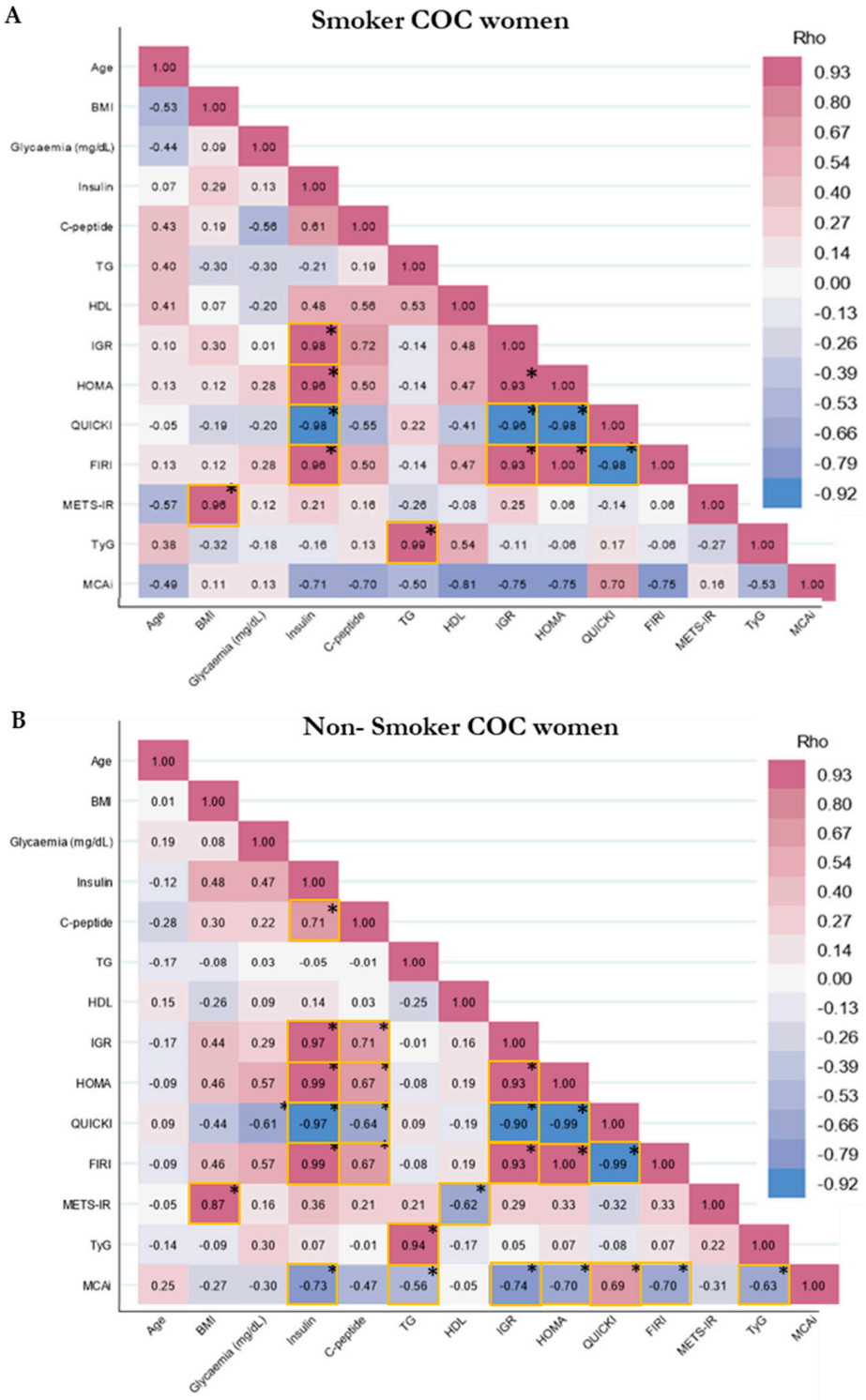

Figure 3. Heat maps of correlations in smoker (**A**) and non-smoker (**B**) COC women. Values represent Spearman's correlation coefficient. * in the yellow boxes represents the statistically significant correlations.

3.3. Intrasex Differences

In men and COC users, none of the analyzed parameters was significantly different between smokers and non-smokers (Table 4).

Table 4. Intrasex analysis in men and COC women.

Variables	Men			COC Women		
	Non-Smoker (*n* = 33)	**Smoker (*n* = 22)**	***p*-Value**	**Non-Smoker (*n* = 43)**	**Smoker (*n* = 13)**	***p*-Value**
Age (years)	27.9 (3.8)	26.9 (5.0)	0.41	27 (25–30)	27 (24–30)	0.87
BMI (kg/m^2)	23.8 (2.4)	23.2 (3.3)	0.46	21.1 (2.1)	22.2 (2.9)	0.16
Glycemia (mgd/L)	82.0 (9.7)	80.6 (10.8)	0.62	76.5 (9.1)	79.2 (5.2)	0.32
Insulin (uU/mL)	6.7(4.9–8.8)	5.5 (3.4–6.8)	0.10	7.1 (5.4–9.1)	7.5 (4.5–7.8)	0.85
C-peptide (ng/mL)	1.7 (0.5)	1.7 (0.5)	0.97	1.7 (1.4–2.3)	1.5 (1.3–2.9)	0.87
TG (md/dL)	75 (58–103)	72.5 (58–102)	0.74	85.5 (70–122)	95 (68–114)	0.89
HDL (mg/dL)	50 (42.5–57.5)	51 (45–62)	0.43	71.5 (14.8)	67.2 (11.7)	0.33
IGR (U/mol)	1.4 (1.1–1.9)	1.1 (0.9–1.5)	0.10	1.7 (1.3–2.1)	1.6 (1.2–1.7)	0.45
HOMA-IR	1.2 (0.9–1.7)	1.1 (0.7–1.4)	0.13	1.3 (0.9–1.7)	1.5 (0.8–1.7)	0.74
QUICKI	0.37 (0.03)	0.39 (0.04)	0.11	0.37 (0.03)	0.37 (0.03)	0.73
FIRI	1.1 (0.8–1.6)	1.0 (0.6–1.3)	0.13	1.2 (0.8–1.5)	1.3 (0.7–1.5)	0.73
METS-IR	32.8 (31.0–35.2)	31.1 (28.7–35.0)	0.17	27.4 (3.2)	29.3 (3.6)	0.09
TyG	4.4 (0.2)	4.4 (0.3)	0.61	4.4 (0.2)	4.5 (0.2)	0.67
MCAi	6.2 (0.5)	6.3 (0.06)	0.47	6.0 (0.4)	6.0 (0.5)	0.97

Data are expressed by mean (SD) or median (IQR).

COC-free women who smoke had higher insulin, C-peptide, TG, and IGR and lower MCAi than non-smokers (Table 5).

Table 5. Intrasex analysis in COC-free women.

Variables	**Non-Smoker (*n* = 32)**	**Smoker (*n*= 24)**	***p*-Value**
Age (years)	28.2 (5.0)	27 (4.4)	0.36
BMI (kg/m^2)	22.4 (4.8)	21.7 (3.9)	0.56
Glycemia (mg/dL)	77.9 (9.8)	74.4 (13.2)	0.27
Insulin (µU/mL)	4.7 (3.9–7.7)	6.4 (4.8–9.6)	**0.04**
C-peptide (ng/mL)	1.4 (1.2–1.6)	2 (1.7–2)	**0.0001**
TG (md/dL)	63.5 (50.5–72.5)	73 (62.5–90)	**0.03**
HDL (mg/dL)	60.9 (9.9)	60.0 (11.7)	0.77
IGR (U/mol)	1.2 (0.9–1.8)	1.6 (1.1–2.5)	**0.03**
HOMA-IR	1.0 (0.7–1.4)	1.2 (0.9–1.6)	0.08
QUICKI	0.39 (0.03)	0.37 (0.03)	0.07
FIRI	0.89 (0.6–1.2)	1.10 (0.8–1.5)	0.08
METS-IR	26.9 (25.5–32.9)	27.7 (24.6–31.4)	0.92
TyG	4.3 (0.2)	4.3 (0.2)	0.16
MCAi	6.5 (0.5)	6.1 (0.4)	**0.003**

Data are expressed by mean (SD) or median (IQR).

3.4. Cluster Analysis

Three main clusters were found (Table 6 and Supplementary Figure S1), with significant separation between sexes and between smokers and non-smokers. Cluster 1 included only non-smokers and they were equally distributed in the three cohorts (Table 6). Cluster 2 included mostly COC women (87.5%) and Cluster 3 included only smokers, with a lower % of COC users (16.3%). In addition, cluster 1 is characterized by lower C-peptide, TG, and higher MCAi in comparison with clusters 2 and 3. BMI, METS-IR, and QUICKI were higher in cluster 1 than in cluster 2; whereas insulin, HDL, IGR, HOMA-IR, FIRI, and TyG were lower than in cluster 2. HDL and IGR were higher in Cluster 2 than in Cluster 3, whereas METS-IR and MCAi were lower in Cluster 2 than in Cluster 3.

Table 6. Cluster analysis.

Variables		Cluster 1 (*n* = 57)	Cluster 2 (*n* = 40)	Cluster 3 (*n* = 49)	*p*-Value
Smoke, n (%)		0 (0.0)	3 (7.5)	49 (100.0)	**<0.0001**
Females, n (%)		29 (50.9)	36 (90.0)	29 (59.2)	**<0.0001**
	COC free women	28 (49.1)	1 (2.5)	21 (42.9)	
Groups, n (%)	Men	28 (49.1)	4 (10.0)	20 (40.8)	**<0.0001**
	Women COC	1 (1.8)	35 (87.5)	8 (16.3)	
Age		28.1 (4.4)	27.9 (3.9)	26.7 (4.6)	0.21
BMI		23.1 (3.8) *	21.3 (2.6)	22.6 (3.7)	**0.04**
Glycemia (mgd/L)		79.9 (8.8)	79.0 (9.6)	78.0 (11.0)	0.62
Insulin (uU/mL)		5.6 (3.9–7.4) *	7.7 (5.5–12.5)	6.1 (4.5–8.2)	**0.004**
C-peptide (ng/mL)		1.5 (1.2–1.8) *,°	1.9 (1.5–2.3)	1.8 (1.5–2)	**0.001**
TG (md/dL)		62 (51–79) *,°	85.5 (74–130)	74 (64–101)	**0.0001**
HDL (mg/dL)		55.8 (12.1) *	69.2 (16.0) ^	60.1 (14.1)	**<0.0001**
IGR (U/mol)		1.3 (1.0–1.8) *	1.8 (1.3–2.6) ^	1.4 (1.0–1.8)	**0.0006**
HOMA-IR		1.1 (0.8–1.6) *	1.4 (1.0–2.6)	1.2 (0.8–1.5)	**0.01**
QUICKI		0.38 (0.03) *	0.36 (0.03)	0.38 (0.03)	**0.002**
FIRI		1.0 (0.7–1.4) *	1.3 (0.9–2.3)	1.1 (0.7–1.4)	**0.01**
METS-IR		31.6 (27.0–33.4) *	26.8 (25.0–29.9) ^	29.89 (26.6–34.2)	**0.005**
TyG		4.3 (0.2) *	4.5 (0.2)	4.4 (0.2)	**0.0003**
MCAi		6.4 (0.4) *,°	5.8 (0.9) ^	6.2 (0.5)	**<0.0001**

Data are expressed as a percentage (%), mean (SD), or median (IQR). Post-hoc comparison: * statistically significant difference between Cluster 1 and Cluster 2; ° between Cluster 1 and Cluster 3; and ^ between Cluster 2 and Cluster 3.

4. Discussion

Tobacco smoking, consumed by millions of persons worldwide [22], can affect several physiological pathways including glucose homeostasis [11]. Previously, it was shown that non-obese healthy young adult women and men have similar insulin sensitivity, but COC use elevates IR associated with abnormal fatty acid metabolism [23,24].

Few research reports some data on the effects of COC [25–27] and sex [28–30] on the M-index measured with a hyperinsulinemic-euglycemic clamp or glucose tolerance tests. Greater attention has been given to investigating the effect of smoking on the M-index [31–33]. However, most of these studies involved subjects with various pathologies, such as PCOS, obesity, or T1D. One study [34] did report a higher M-value in apparently healthy Asian women compared to men (with an age range of 21–65 years).

However, to the best of our knowledge, the effects of the combination of tobacco and COC use [35] on surrogate indexes of IR were unknown.

Before smoking stratification, our data show that fasting insulin, C-peptide, HOMA-IR, QUICKI, and FIRI do not diverge in COC-users versus COC-free women while HDL, TG, and TyG are higher and MCAi is lower in COC-users than in COC-free women. Thus, COC can affect the female phenotype. Fasting glycemia, BMI, TG, and METS-IR are higher and HDL is lower in men than in COC-free-women, while IGR is lower and MCAi and METS-IR are higher in men than in COC-users. Notably, the variability of MCAi and TyG are relevant, being a predictive and prognostic biomarker for cardiovascular morbidity and mortality [36,37] and a predictor of diabetes and CVD in healthy and hypertensive individuals associated with arterial stiffness, respectively [38]. Moreover, higher levels of METS-IR are predictive of future ischemic heart disease [39].

IR is higher in men than in COC-free women as proved by several indexes [40]. A previous study did not find a relationship between HOMA-IR and IGR with sex and gender [41], whereas another study showed higher HOMA-IR in Spanish men than in women aged 20–90 years [42]. No sex or gender differences were found in METS-IR in subjects aged >65 years [43], whereas a direct correlation between METS-IR and the risk of prediabetes was described in Chinese men and women with normal blood glucose, with a

stronger correlation in women [44]. Some studies reported a relationship between the COC and IR indexes, with COC affecting carbohydrate metabolism [45].

Looking at BMI, it is not correlated with parameters in COC-free women but is positively correlated with insulin, HOMA-IR, FIRI, and IGR and negatively with QUICKI in COC-users, suggesting their sensitivity to the hormonal milieu.

Smoking stratification reduces the differences between men and women. In particular, HDL is higher in COC smokers than in men and IGR is higher in smokers COC-free women than men. Interestingly, in COC-free women, smoking causes a significant increase in basal insulin secretion (insulin and C-peptide significantly higher) with a simultaneous non-significant increase in HOMA-IR, a significant increase in IGR, and a decrease in MCAi, as if smoking induces an increase in IR, supported by an increased insulin secretion. This could occur in insulin secretion deficiency (typical of the female sex), increasing the risk of type 2 diabetes. This aspect is not observed in men and COC users. It is therefore clear that there is a need for more monitoring of this specific population (COC-free women), which is often not enrolled in clinical trials, in which the use of a contraceptive is notoriously required.

Conflicting results on tobacco smoking and IR have been published: some authors did not find a significant relationship in healthy individuals [46] while Gupta et al. reported a higher HOMA-IR in male smokers aged 18–40 years [17]. Finally, others showed that cigarette smoking is associated with an increased METS-IR, especially among individuals aged <70 years [47].

A similar effect of smoking and COC use was found when inflammatory and atherosclerotic indexes were evaluated [14,15]. In both cases, tobacco smoke reduced the differences observed in men and women and highlighted the differences between COC users and COC-free women. In this study, the cluster analysis has more clearly shown the interaction between smoking and COC use.

Interestingly, in our cohort, COC users had more similarities with men than with COC-free women, indicating the need to study them separately. The combination of COC and smoking requires more monitoring because it increases diabetes and cardiovascular risk through several pathophysiological mechanisms (inflammation, atherosclerosis, IR), as well as the thrombotic risk already extensively described in the past [16].

Given the large use of tobacco and COC [22,35], their combination could be a public health problem.

In conclusion, although larger studies are necessary to conclude this topic, the study evidenced that smoking can be associated with changes in the phenotype of both healthy men and women; interestingly, women's phenotype is modified by the use of COC.

Therefore, smoking and the use of COC should be considered two independent variables in clinical studies given their effects on phenotype. This is fundamental to arrive at a personalization of prevention and care.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/diabetology5070050/s1: Figure S1. Dendrogram illustrating hierarchical cluster analysis of 146 patients using Gower distance, with three distinct clusters represented by different colors (blue, green, and yellow).

Author Contributions: G.T.: samples collection and laboratory examinations, manuscript writing, review, and editing; A.M.: manuscript review and editing; M.V.P., G.S. (Giovanni Sotgiu), and N.M.: statistical analysis, manuscript review and editing; S.C.: samples collection; M.P.: manuscript review and editing; G.S. (Giuseppe Seghieri): manuscript review and editing; F.F.: Investigation, manuscript review and editing; I.C.: Conceptualization, investigation, validation, writing original draft, and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partly funded by Fondazione di Sardegna through the grant "Bando competitivo Fondazione di Sardegna—2017" and with the support of SIMDO (Societa' Italiana Metabolismo Diabete E Obesita').

Institutional Review Board Statement: The study was approved by the Ethical Committee of AOU Cagliari (prot. PG/2019/6280) and informed consent was obtained from all subjects. All methods were carried out according to relevant guidelines and regulations.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data will be made available on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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