# From Metabolic Normality to Cardiometabolic Risk Factors in Subjects With Obesity

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**The aim of the study was to evaluate the 3 years incidence of cardiometabolic risk factors, such as impaired fasting glucose, reduced high-density lipoprotein (HDL)-cholesterol, increased plasma triglycerides or blood pressure as well as impaired glucose tolerance in overweight or obese (ow/ob) and normal body weight (nbw) subjects metabolically normal at baseline. Subjects from the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study were analyzed. We analyzed 284 nbw and 152 ow/ob subjects who, at baseline, did not show any of the above-mentioned cardiometabolic risk factors. At 3 years, these parameters were re-evaluated. Intima-media thickness (IMT) of the common carotid artery (CCA) was echographically measured. At follow-up, the incidence of one or more cardiometabolic risk factors was 57.2% in ow/ob vs. 31.7% in nbw ($P < 0.0001$). After adjustment for age, sex, menopause status, lifestyle parameters, insulin sensitivity, and fasting insulinemia, BMI remained significantly linked to the development of one or more cardiometabolic risk factors ($P = 0.02$). An increased BMI at follow-up was significantly associated with the development of cardiometabolic alterations, in both nbw and ow/ob groups ($P = 0.04$). Ow/ob subjects who, at 3 years follow-up, remained metabolically normal, showed a less favourable cardiometabolic profile, when compared to nbw counterparts. In ow/ob metabolically normal males and females, intima-media of the common carotid at follow-up was thicker than in nbw ($P = 0.03$ for males, $P = 0.04$ for females). In conclusion, metabolically normal obese subjects show a higher incidence of cardiometabolic risk factors, in a short follow-up period. Weight gain is significantly associated with the development of these factors, in both nbw and ow/ob subjects.**

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## INTRODUCTION

Metabolic normality in obese subjects (MNO) has been differently defined across numerous studies (1,2). It is generally agreed that uncomplicated obesity indicates a subset of obese subjects who do not show any of the obesity-related cardiometabolic diseases. Furthermore, MNO do not present any risk factor for these pathologies, such as the ones clustering for metabolic syndrome (3). Insulin sensitivity has also been included among the parameters contributing to the definition of metabolic normality (4,5) because of its potential role in the development of the obesity-related cardiometabolic alterations (6). Beside the need of an expert consensus about the definition of metabolic normality, the major question that remains open is whether healthy obese will remain so or will evolve through the appearance of risk factors for cardiometabolic diseases and, then, the development of overt pathologies. Answering this question is of primary importance, since it will determine the strategies, in terms of surveillance and/or treatment, which have to be adopted in the management of MNO. To our knowledge, only few studies have dealt with this relevant question: two of them (7,8) have demonstrated a substantially increased incidence of diabetes, in MNO. In another, a 9-year follow-up study, Kuk and Ardern (9) showed an increased risk for all-cause mortality, in obese subjects regardless if they were metabolically normal or not, whereas Arnlöv *et al.* (10) found higher risk for cardiovascular disease, in MNO. In these studies, metabolic normality was based either on the absence of metabolic syndrome, as defined by the ATP III criteria (11), or on the presence of a normal insulin sensitivity, as evaluated by homeostatic model assessment (HOMA) index (12). In a recent study, Calori *et al.* (13) demonstrated that obese, insulin resistant subjects show an increased risk for all-cause mortality and, in particular, for cardiovascular disease and cancer-related mortality. In this investigation, the definition of metabolic normality was restricted to the presence of insulin resistance, as evaluated by the HOMA index and only obese subjects were

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evaluated. In absence of normal body weight (nbw) control groups, either insulin sensitive and insulin resistant, it is difficult to know whether the observed increased mortality is linked to obesity or to insulin resistance.

In a previous study (14), we demonstrated that, among clinically healthy overweight or obese (ow/ob) subjects, 37% were free from cardiometabolic risk factors, such as those clustering in the metabolic syndrome, and with a normal glucose tolerance and only 11% also showed normal insulin sensitivity and fasting plasma insulin. Ow/ob subjects from both subgroups displayed a less favourable cardiometabolic profile, thus suggesting that they could be at increased risk for cardiometabolic diseases. In order to examine this hypothesis, we evaluated, in the present follow-up study, the incidence of cardiometabolic risk factors in this population of ow/ob subjects who, at baseline, were free from these alterations.

## METHODS AND PROCEDURES

### Study group

As previously stated, our study group was selected among the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study cohort. The rationale and design of the RISC study has been previously published (15). In brief, participants were recruited from the local population at 21 centres in 14 European countries, according to the following inclusion criteria: either sex, age between 30 and 60 years, and clinically healthy. Exclusion criteria were the presence of chronic diseases, overt cardiovascular disease, carotid stenosis >40%, and treatment for hypertension, diabetes, and dyslipidemia. Moreover, after screening, only subjects ($n$ = 1,227, 619 nbw and 608 ow/ob) with blood pressure <140/90 mm Hg, plasma cholesterol <7.8 mmol/l, triglycerides <4.6 mmol/l, and fasting and 2-h glucose <7.0 and 11.1 mmol/l, respectively were enrolled.

Among this cohort, we selected subjects who, at baseline, showed a normal glucose tolerance and were free from cardiometabolic risk factors. Namely, all the selected subjects had fasting glucose <5.6 mmol/l, fasting triglycerides <1.7 mmol/l, high-density lipoprotein (HDL) cholesterol >1.03 and >1.29 mmol/l, respectively in men and women, blood pressure <130/85 mm Hg and plasma glucose levels <7.8 mmol/l 2 h after a 75-g oral glucose tolerance test. Participants taking, at baseline, treatment for diabetes, dyslipidaemia or hypertension were not included in the study. Waist circumference was not taken into account for the selection, neither in ow/ob or in nbw group. Finally, we included only subjects who performed the 3-year follow-up study. This last consisted in the re-evaluation of the following parameters: body weight, plasma glucose, and insulin both in fasting conditions and after oral glucose load, total, low-density lipoprotein (LDL) and HDL-cholesterol as well as triglycerides. Systolic and diastolic blood pressure and the intima-media thickness (IMT) of the common segment of the carotid artery were also measured.

The selection yielded 436 subjects who were classified according to their BMI (i.e., body weight/height$^2$) as normal body weight (nbw, i.e., BMI <25 kg/m$^2$, $n$ = 284, 76 males and 208 females, mean age 43 ± 8 years) or overweight-obese (ow/ob, i.e., BMI ≥25 kg/m$^2$, $n$ = 152, 66 males and 86 females, mean age 45 ± 9 years). In these subjects, we investigated the 3-year follow-up incidence of cardiometabolic risk factors, as defined by the International Diabetes Federation (IDF) (16) and of impaired glucose tolerance, i.e., plasma glucose levels ≥7.8 mmol/l 2 h after a 75-g oral glucose load. Subjects taking medication for diabetes, dyslipidemia, or hypertension at follow-up were included in the group of subjects "at-risk."

The IMT of the carotid artery at the level of the common segment was measured, at 3-year follow-up, in 376 subjects, physical activity and alcohol consumption was evaluated in 424 participants and tobacco consumption in 394.

The study protocol was approved by the local ethical committee. Participants were informed about the aims of the study and gave their written consent.

### Insulin sensitivity

Insulin sensitivity was evaluated by euglycemic hyperinsulinaemic clamp (17), at baseline. Insulin was continuously infused at a rate of 240 pmol/min/m$^2$, whereas a glucose solution (20%) was infused at variable rates in order to maintain a constant plasma glucose concentration between 4.5 and 5.5 mmol/l. Plasma glucose was measured at 5–10 min intervals to ensure it remained within the target glucose concentration. The steady-state period (for calculation of insulin sensitivity) was between 80 to 120 min. Glucose uptake, measured during the steady-state period, was expressed in mmol/kg fat-free mass/min/pmol insulin. Fat-free mass was measured by TANITA bioimpedance balance (Tanita International Division, Yiewsley, Middlesex, UK).

### Carotid artery ultrasound imaging and blood pressure measurements

Carotid artery intima-media imaging, performed both at baseline and follow-up, followed a validated protocol (18). Longitudinal B-mode image was taken of the right and left common carotid arteries (CCA) from anterior, lateral, and posterior angles. The images from all the participating centres were evaluated by a single reader blind to the weight status. Diastolic frames of CCA were selected to provide images of the near and far wall intima-media complex. Frames were digitized and analyzed by an image analysis system (19) (MIP: Institute of Clinical Physiology, CNR, Pisa, Italy). Lines were drawn along the lumen-intimal and medial-adventitial interfaces, and the IMT was computed as an average of two measurements. A 30-s longitudinal B-mode image was made of the right CCA and concomitant blood pressure was measured using a sphygmomanometric cuff. An automatic contour detection algorithm determined the average minimum and maximum CCA diameter.

Sitting blood pressure and heart rate are the mean values of three measurements (OMRON 705 cp; OMRON Healthcare, Kyoto, Japan).

### Analytical methods

Plasma samples were centralized in different Laboratories. In Odense, Denmark, glucose was determined by the glucose oxidase technique (Cobas Integra; Hoffmann La-Roche, Basel, Switzerland), insulin and c-peptide by specific by a two-site time-resolved fluroimmunoassay (AutoDELFIA Insulin kit; Wallac Oy, Turku, Finland). Plasma adiponectin was determined by a previously described time-resolved immunofluorometric assay (20). In Dublin, Ireland, total and HDL and triglycerides were measured by Roche enzymatic colorimetric methods for Modular Systems (Hoffmann La-Roche). LDL concentration was calculated by the Friedewald formula (21).

### Lifestyle parameters assessment

Information was collected on socioeconomic status of the participants and their partners, personal medical history and family history of cardiovascular disease, stroke, hypertension and diabetes in first-degree relatives, smoking and alcohol habits, and physical activity. The lifestyle questionnaire was prepared in English, translated into the 11 languages of the study and back-translated into English to ensure homogeneity.

Qualitative information on physical activity was collected with the 7-day International Physical Activity Questionnaire (IPAQ), a previously validated assessment tool for international studies (22). The specific activities performed in various settings were coded and transformed on respective metabolic equivalents according to previously published and validated coding scheme (23).

### Statistical analysis

The incidence of cardiometabolic risk factors and impaired glucose tolerance in ow/ob vs. nbw subjects was analyzed by $\chi^2$-test.

The independent contributors to the development of these alterations were investigated by multiple regression analysis. Differences between subgroups were evaluated by factorial ANOVA followed by Fisher's *post-hoc* test. The analyses were performed after adjustment for study centres, results are expressed as mean ± SD. Because of the non normal distributions of some parameters, values were statistically evaluated after log transformation.

## RESULTS

### Characteristics of the study groups

Tables 1 and 2 summarize anthropometrical and cardiometabolic characteristics of the studied population who was metabolically normal at baseline (e.g., absence of cardiometabolic risk factors with the exception of increased waist circumference), respectively in male and female subjects, both nbw and ow/ob.

### Comparison between at-risk vs. metabolically normal subjects within each BMI class

In males (Table 1), at the 3-year follow-up, both nbw and ow/ob male subjects who developed ≥1 cardiometabolic risk factors during the follow-up showed increased fasting and 2-h glucose levels and increased blood pressure values as compared to metabolically normal group. Moreover, metabolically at-risk males at follow-up showed increased triglycerides, LDL-cholesterol and fasting insulin in the nbw subgroup and decreased HDL-cholesterol levels in the ow/ob subgroup when compared to metabolically normal subjects.

In females (Table 2), at the 3-year follow-up, the subjects who became metabolically at-risk at follow-up showed higher fasting and 2-h glucose, triglycerides, and blood pressure as compared to metabolically normal subjects. In the nbw subgroup, higher total and LDL-cholesterol and lower HDL-cholesterol levels were observed in the metabolically at-risk group.

In both nbw males and females, subjects at-risk showed an increased CCA-IMT, at follow-up.

### Comparison of 3-year values between metabolically normal ow/ob and nbw subjects

In males (Table 1), ow/ob who remained metabolically normal at follow-up showed higher fasting insulinemia and LDL-cholesterol as compared to nbw controls.

In females (Table 2), ow/ob group showed higher fasting and 2-h glucose, plasma triglycerides and LDL-cholesterol as well as decreased HDL-cholesterol. Fasting insulinemia and CCA-IMT were also significantly increased in ow/ob group.

### Comparison of 3-year values between at-risk ow/ob and nbw subjects

In metabolically at-risk females (Table 2), fasting glucose and insulinemia as well as systolic blood pressure and diastolic blood pressure were significantly higher, in comparison to at-risk nbw group.

### Comparison of baseline values between nbw who, at follow-up, remained metabolically normal or developed risk(s) factor(s)

Among male nbw group (Table 1), subjects who developed risk(s) factor(s) at follow-up, presented, at baseline, increased values of total and LDL-cholesterol, whereas nbw females (Table 2) had higher triglycerides, total cholesterol, systolic and diastolic blood pressure. CCA-IMT was also increased in females who developed risk(s) factor(s) at follow-up

**Table 1 Baseline and 3-year data of male subjects who, being metabolically normal at baseline, remained so or developed ≥1 cardiometabolic risk factors at follow-up**

| | nbw | | | | ow/ob | | | |
|---|---|---|---|---|---|---|---|---|
| | Metabolically normal at follow-up (*n* = 45) | | Metabolically at-risk at follow-up (*n* = 31) | | Metabolically normal at follow-up (*n* = 27) | | Metabolically at-risk at follow-up (*n* = 39) | |
| | Baseline | 3 years | Baseline | 3 years | Baseline | 3 years | Baseline | 3 years |
| BMI (kg/m$^2$) | 22.7 ± 1.5 | 23.0 ± 1.6 | 22.7 ± 1.7 | 23.1 ± 2.0 | 26.8 ± 1.4 | 26.8 ± 1.8$^{b,***}$ | 27.8 ± 2.3 | 27.9 ± 2.6$^{b,***}$ |
| Waist circumference (cm) | 82.9 ± 5.4 | 84.9 ± 6.0 | 85.3 ± 5.8 | 85.7 ± 6.6 | 93.1 ± 8.8 | 93.4 ± 9.4$^{b,***}$ | 98.1 ± 8.3 | 97.7 ± 8.3$^{b,***}$ |
| Fasting glucose (mmol/l) | 5.0 ± 0.3 | 4.9 ± 0.7 | 5.0 ± 0.3 | 5.3 ± 0.5$^{a,*}$ | 5.0 ± 0.4 | 5.0 ± 0.4 | 5.2 ± 0.3 | 5.4 ± 0.4$^{a,***}$ |
| 2-h Glucose (mmol/l) | 4.7 ± 1.0 | 4.9 ± 1.1 | 5.0 ± 1.2 | 5.6 ± 1.1$^{a,**}$ | 5.5 ± 0.9 | 5.4 ± 0.8 | 5.5 ± 1.1 | 6.2 ± 1.8$^{a,*}$ |
| Triglycerides (mmol/l) | 0.86 ± 0.29 | 0.85 ± 0.29 | 0.91 ± 0.29 | 1.02 ± 0.41$^{a,*}$ | 0.95 ± 0.23 | 0.93 ± 0.26 | 0.96 ± 0.29 | 1.17 ± 0.55 |
| HDL (mmol/l) | 1.5 ± 0.3 | 1.5 ± 0.3 | 1.4 ± 0.2 | 1.4 ± 0.4 | 1.4 ± 0.2 | 1.4 ± 0.2 | 1.3 ± 0.2$^{c,*}$ | 1.3 ± 0.3$^{a,*}$ |
| SBP (mm Hg) | 115 ± 8 | 117 ± 8 | 118 ± 8 | 126 ± 17$^{a,*}$ | 117 ± 6 | 118 ± 9 | 120 ± 8 | 126 ± 10$^{a,*}$ |
| DBP (mm Hg) | 72 ± 7 | 71 ± 7 | 75 ± 6 | 77 ± 12$^{a,*}$ | 73 ± 6 | 74 ± 7 | 74 ± 6 | 80 ± 7$^{a,*}$ |
| Fasting insulin (pmol/l) | 19.8 ± 9.2 | 21.9 ± 12.6 | 25.1 ± 13.3 | 32.8 ± 17.6$^{a,**}$ | 28.7 ± 10.1 | 33.7 ± 14.4$^{b,**}$ | 27.8 ± 13.0 | 38.2 ± 19.6 |
| Total cholesterol (mmol/l) | 4.6 ± 0.8 | 4.6 ± 0.8 | 5.0 ± 0.8$^{c,*}$ | 5.1 ± 1.0 | 5.0 ± 0.9 | 4.8 ± 0.8 | 4.9 ± 0.8 | 5.0 ± 0.9 |
| LDL (mmol/l) | 2.7 ± 0.7 | 2.7 ± 0.6 | 3.2 ± 0.7$^{c,**}$ | 3.2 ± 0.8$^{a,*}$ | 3.2 ± 0.8 | 3.1 ± 0.7$^{b,**}$ | 3.2 ± 0.8 | 3.2 ± 0.8 |
| CCA-IMT (mm) | 0.59 ± 0.08 | 0.60 ± 0.07 | 0.61 ± 0.09 | 0.63 ± 0.08$^{a,*}$ | 0.63 ± 0.07 | 0.62 ± 0.10 | 0.65 ± 0.09 | 0.67 ± 0.1 |

Metabolically normal subjects: No risk factors with the exception of increased waist circumference at 3 years. Metabolically at-risk subjects: ≥1 cardiometabolic risk factors at 3 years. Results are expressed as mean ± SD.

CCA-IMT, common carotid artery-intima-media thickness; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; nbw, normal body weight; ow/ob, overweight-obese; SBP, systolic blood pressure.

[a]vs. metabolically normal subgroup at 3 years. [b]vs. nbw subgroup at 3 years. [c]vs. metabolically normal subgroup at baseline.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

**Table 2 Baseline and 3-year data of female subjects who, being metabolically normal at baseline, remained so or developed ≥ 1 cardiometabolic risk factors at follow-up**

| | nbw | | | | ow/ob | | | |
|---|---|---|---|---|---|---|---|---|
| | Metabolically normal at follow-up ($n$ = 149) | | Metabolically at-risk at follow-up ($n$ = 59) | | Metabolically normal at follow-up ($n$ = 38) | | Metabolically at-risk at follow-up ($n$ = 48) | |
| | Baseline | 3 years | Baseline | 3 years | Baseline | 3 years | Baseline | 3 years |
| BMI (kg/m$^2$) | 21.7 ± 1.9 | 21.9 ± 2.1 | 22.1 ± 0.7 | 22.7 ± 2.1$^{a,*}$ | 27.6 ± 2.5 | 27.8 ± 3.1$^{b,***}$ | 28.5 ± 3.8 | 29.7 ± 4.7$^{a,**,b,***}$ |
| Waist circumference (cm) | 74.1 ± 7.3 | 74.8 ± 6.9 | 75.1 ± 5.8 | 76.8 ± 7.8$^{a,*}$ | 85.9 ± 8.1 | 87.2 ± 9.5$^{b,***}$ | 89.2 ± 10.7$^{c,*}$ | 91.6 ± 11.3$^{a,**,b,**}$ |
| Fasting glucose (mmol/l) | 4.8 ± 0.4 | 4.8 ± 0.5 | 4.8 ± 0.3 | 5.0 ± 0.6$^{a,**}$ | 4.9 ± 0.3 | 4.9 ± 0.4$^{b,*}$ | 5.0 ± 0.5 | 5.6 ± 1.0$^{a,***,b,***}$ |
| 2-h Glucose (mmol/l) | 5.2 ± 1.1 | 5.1 ± 1.1 | 5.5 ± 1.3 | 5.8 ± 1.5$^{a,***}$ | 5.5 ± 1.0 | 5.4 ± 1.1$^{b,*}$ | 5.7 ± 1.1 | 6.4 ± 1.3$^{a,***}$ |
| Triglycerides (mmol/l) | 0.73 ± 0.26 | 0.72 ± 0.24 | 0.85 ± 0.33$^{c,**}$ | 1.09 ± 0.60$^{a,***}$ | 0.86 ± 0.28 | 0.89 ± 0.28$^{b,***}$ | 0.92 ± 0.33 | 1.18 ± 0.60$^{a,**}$ |
| HDL (mmol/l) | 1.8 ± 0.3 | 1.8 ± 0.3 | 1.7 ± 0.3 | 1.6 ± 0.5$^{a,***}$ | 1.6 ± 0.2 | 1.7 ± 0.3$^{b,***}$ | 1.6 ± 0.3 | 1.5 ± 0.4 |
| SBP (mm Hg) | 107 ± 10 | 108 ± 10 | 111 ± 10$^{c,*}$ | 117 ± 14$^{a,***}$ | 112 ± 9 | 111 ± 9 | 117 ± 7$^{c,**}$ | 124 ± 15$^{a,***,b,*}$ |
| DBP (mm Hg) | 68 ± 7 | 69 ± 7 | 72 ± 7$^{c,**}$ | 74 ± 9$^{a,***}$ | 71 ± 6 | 70 ± 6 | 75 ± 6$^{c,*}$ | 80 ± 8$^{a,***,b,**}$ |
| Fasting insulin (pmol/l) | 21.8 ± 10.5 | 25.2 ± 10.4 | 23.6 ± 8.6 | 27.6 ± 18.2 | 29.3 ± 14.1 | 36.0 ± 20.9$^{b,***}$ | 31.4 ± 15.0 | 43.4 ± 20.2$^{b,***}$ |
| Total cholesterol (mmol/l) | 4.6 ± 0.8 | 4.7 ± 0.8 | 5.0 ± 0.7$^{c,*}$ | 5.0 ± 0.8$^{a,**}$ | 4.9 ± 0.8 | 5.0 ± 0.9 | 5.0 ± 0.7 | 5.1 ± 0.8 |
| LDL (mmol/l) | 2.5 ± 0.7 | 2.5 ± 0.7 | 2.9 ± 0.6 | 2.9 ± 0.7$^{a,***}$ | 2.9 ± 0.8 | 3.0 ± 0.8$^{b,**}$ | 2.9 ± 0.6 | 3.0 ± 0.7 |
| CCA-IMT (mm) | 0.56 ± 0.06 | 0.58 ± 0.06 | 0.59 ± 0.08$^{c,*}$ | 0.61 ± 0.08$^{a,*}$ | 0.57 ± 0.06 | 0.61 ± 0.08$^{b,*}$ | 0.60 ± 0.08 | 0.63 ± 0.08 |

Metabolically normal subjects: no risk factors with the exception of increased waist circumference at 3 years. Metabolically at-risk subjects: ≥1 cardiometabolic risk factors at 3 years. Results are expressed as mean ± SD.
CCA-IMT, common carotid artery-intima-media thickness; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; nbw, normal body weight; ow/ob, overweight-obese; SBP, systolic blood pressure.
[a]vs. metabolically normal subgroup at 3 years. [b]vs. nbw subgroup at 3 years. [c]vs. metabolically normal subgroup at baseline.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

### Comparison of baseline values between ow/ob who, at follow-up, remained metabolically normal or developed risk(s) factor(s)

Baseline HDL-cholesterol was significantly lower in ow/ob male subjects who developed risk(s) factors at follow-up (Table 1), whereas systolic and diastolic blood pressure were significantly increased in the corresponding female group (Table 2).

### Incidence of metabolic alteration at follow-up

Figure 1 illustrates the 3-year incidence of one or more cardiometabolic risk factors in ow/ob and nbw subjects who, at baseline, were free from any metabolic alterations. During the follow-up, 57.2% of ow/ob subjects and 31.7% of nbw subjects developed ≥1 cardiometabolic risk factor ($\chi^2$ $P$ value <0.0001).

In addition, males were more prone to develop cardiometabolic risk factors than females (49.3% vs. 36.4%, $P = 0.01$).

### Contributors to the development of one or more cardiometabolic risk factors at follow-up

In multiple regression analysis (Table 3), the role of baseline BMI in the occurrence of cardiometabolic alterations was analyzed together with the following independent variables also measured at baseline: sex, age, menopause status, waist circumference, insulin sensitivity and fasting insulinemia as well as physical activity and both tobacco and alcohol consumption.

Beside sex, only BMI ($P = 0.02$) was significantly associated to the occurrence of ≥1 cardiometabolic risk factors, at 3-year follow-up.

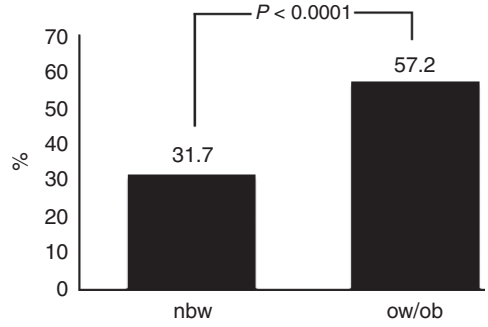


**Figure 1** Incidence of ≥1 cardiometabolic risk factors at 3-year follow-up. nbw, normal body weight subjects; ow/ob, overweight or obese subjects.

### Association between modifications of BMI and metabolic normality at follow-up

As indicated in Figure 2, the participants were classified according to the evolution of their cardiometabolic profile during the follow-up. The ΔBMI was calculated as the difference between the 3 years and baseline values. Nbw and ow/ob were separately analyzed (Figure 2a and b). In both BMI categories, subjects who did not develop cardiometabolic abnormalities at follow-up (i.e., 194 nbw and 65 ow/ob) served as control group.

Subjects who developed ≥1 cardiometabolic alterations during the follow-up showed significantly larger BMI increase in comparison to the group whose metabolic profile remained unchanged. Namely, in nbw, ΔBMI was 0.20 ± 1.28 in control group vs. 0.59 ± 1.32 kg/m$^2$ in metabolically at-risk group

**Table 3 Multiple regression analysis of baseline contributors to the development ≥1 cardiometabolic risk factors at 3-year follow-up**

| Independent variable/Dependent variable | Development of ≥ 1 cardiometabolic risk factors | |
|---|---|---|
| | Coefficient | *P* |
| Sex | –0.1941 | 0.004 |
| Age | 0.0071 | ns (0.07) |
| Menopause status | 0.1056 | ns |
| BMI | 0.0244 | 0.02 |
| Waist circumference | –0.0086 | ns |
| Insulin sensitivity | –0.0006 | ns |
| Fasting insulinemia | 0.0013 | ns |
| Physical activity | –0.0002 | ns |
| Tobacco consumption | 0.0016 | ns |
| Alcohol consumption | –0.0001 | ns |

ns, not significant

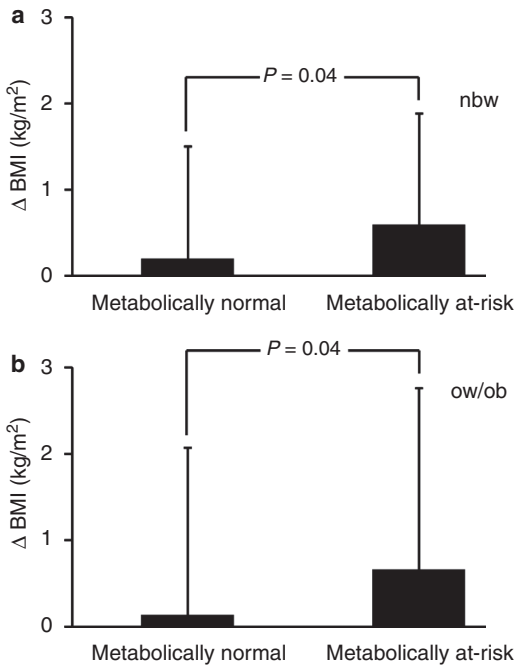


**Figure 2** Delta BMI in metabolically normal and metabolically at-risk subjects at 3 years follow-up in (**a**) normal body weight (nbw) and in (**b**) overweight/obese (ow/ob) subjects. Metabolically normal—no risk factors with the exception of increased waist circumference; metabolically at-risk: ≥1 cardiometabolic risk factors.

($P = 0.04$) and, in ow/ob, ΔBMI was 0.14 ± 1.93 in control group vs. 0.66 ± 2.10 kg/m² in metabolically at-risk group ($P = 0.04$).

## DISCUSSION

As a main result, our study demonstrates that, among ow/ob subjects who, at baseline, were metabolically normal, 57.2% developed one or more cardiometabolic risk factors, 3 years later. This elevated incidence, within a relatively short follow-up period, does not support the idea that metabolically normal obese persons are protected from the risk of cardiometabolic alterations. In addition, an increase in BMI during the follow-up period is significantly associated, in both nbw and ow/ob persons, with the occurrence of cardiometabolic alterations. This further underlines the prominent role played by increased body weight in the development of these alterations. It should be noted that, among nbw subjects who developed one or more cardiometabolic risk factors ($n = 90$), only 14 (i.e., 17%) changed BMI category, from nbw to ow/ob. Despite to that, this increase in BMI was significantly ($P = 0.04$) associated with the development of one or more risk factors, in nbw. This strongly suggests that weight gain *per se* (even when remaining in the nbw class), rather than the transition to the ow/ob category, is determinant in the development of cardiometabolic risk factors. Interestingly, among nbw subjects who developed cardiometabolic risk factors, baseline adiponectin was lower in males group and the frequency of family history of diabetes was significantly higher in females group, as compared to their respective counterparts who remained metabolically normal at follow-up (adiponectin: 7.9 ± 3.5 vs. 6.4 ± 2.2 μg/ml, $P = 0.03$ and family history of diabetes: 38.3% vs. 22.9%, $P = 0.04$).

In a previous study (14), we demonstrated that increased BMI, even in presence of metabolic normality, was independently associated with higher levels of blood pressure, fasting glucose and plasma lipid profile as well as lower HDL-cholesterol. We also showed that both insulin sensitivity and fasting hyperinsulinemia partially contribute to this profile. We, therefore, suggested that ow/ob subjects, even when metabolically normal, could be at increased risk for cardiometabolic alterations. With the present follow-up study, we support our hypothesis by demonstrating the major role of BMI in the occurrence of cardiometabolic alterations. As mentioned in the introduction, the follow-up studies performed in metabolically normal obese (7–10,13) demonstrated an increased risk of diabetes, cardiovascular disease and mortality. To our knowledge, we report for the first time an increased incidence of cardiometabolic risk factors, in metabolically normal ow/ob, in a longitudinal follow-up study.

Finally, ow/ob women who, at follow-up did not develop the mentioned cardiometabolic risk factors (i.e., ~44% of the initial group) and, therefore, remain within the limits of normality, showed significantly higher values of fasting and 2-h glucose, LDL-cholesterol and triglycerides as well as lower HDL-cholesterol as compared to their nbw counterparts. In spite of the fact that the cardiometabolic markers remain within the normal values, the CCA-IMT, considered as an early indicator of atherosclerosis, is also increased in the ow/ob group, as compared to nbw. Two hypotheses can be proposed, in order to explain this observation: either the less favorable cardiometabolic profile, although normal, is already able to negatively influence the development of early atherosclerosis, or ow/ob women are bearing some other risk factors acting independently from the well established ones.

In our study, we included subjects with enlarged waist in the metabolically normal category of both nbw and ow/ob

subgroups. Since the majority of the subjects with enlarged waist belonged to the ow/ob category, this could contribute to the increased incidence of cardiometabolic risk factors, in this group. This hypothesis is not supported by the results of multivariate analysis which showed that BMI remained a significant contributor, independently of the presence of enlarged waist.

It should be noted that, in our study group, ow/ob, and in particular women, were slightly older that nbw controls. This could have somehow contributed to the impairment of the cardiometabolic parameters. On the other hand, none of the lifestyle parameters were significantly different among the subgroups (data not shown), thus eliminating the bias due to these factors. Another limitation of our study consisted in the fact that we did not include insulin sensitivity and fasting insulinemia as criteria for metabolic normality. This was due to the very limited number of participants, among ow/ob, corresponding to these definition (i.e., only 61 subjects).

The questions that remain open are: which therapeutical procedure (if any) should be applied to ow/ob subjects, while they are still metabolically normal? Would a pharmacological approach be justified, despite the normality of the biological markers? Would a reduction of body weight prolong metabolic normality or even make it permanent? There is no doubt that weight loss in obese subjects leads to improvement of cardiometabolic profile and consequent reduction of morbidity and mortality. For instance, in a large cohort of the Diabetes Prevention Program, a modest weight loss was associated with a 34% reduction of diabetes incidence during a 10-year follow-up (24). However, limited literature exists in the field of weight loss benefits in metabolically normal obese subjects. Shin *et al.* could demonstrate a beneficial effect of a 12-week weight loss program in metabolically at-risk obese subjects but not in metabolically normal ones (25). Similarly, Janiszewski *et al.* obtained improvement of cardiometabolic parameters of at-risk obese subjects after weight reduction, but the effects observed in metabolically normal group were more than modest (26). In another study, Karelis *et al.* found an unexpected impairment of insulin sensitivity after weight loss (27). Therefore, long-term follow-up studies conducted in large cohorts are needed in order to demonstrate the beneficial effect of weight reduction, in metabolically normal obese subjects. Meanwhile, our results indicate that metabolically normal obese subjects need a close surveillance of their cardiometabolic parameters as well as a prevention of further weight gain.

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## DISCLOSURE

The authors declared no conflict of interest.
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