**Effect of treatment of periodontitis on incretin axis in obese and non-obese individuals:**

**a cohort study.**

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## Abstract

*Context.*          Periodontitis confers an increased risk of developing type 2 diabetes and, in patients with obesity, it might interfere with the incretin axis. The effect of periodontal treatment on glucoregulatory hormones remains unknown.

*Objective*.          To evaluate the effect of periodontal treatment on incretin axis in obese and lean non-diabetic individuals.

*Setting.*          King's College Dental Hospital and Institute, London, UK.

*Participants and Methods.*          The metabolic profile of obese and BMI-normal individuals affected by periodontitis was studied at baseline, 2 and 6 months after intensive periodontal treatment, by measuring plasma insulin, glucagon, GLP-1 and GIP and markers of systemic inflammation and oxidative stress.

*Main Outcome Measure(s).*    Circulating levels of incretins and inflammatory markers.

*Results.*          At baseline, periodontal parameters were worse for obese than non-obese; this was accompanied by higher levels of circulating hs-CRP, insulin and GLP-1. The response to periodontal treatment was less favourable in the obese group, without significant variations of hs-CRP or malondialdehyde. Gluco-regulatory hormones changed differently after treatment: while insulin and glucagon did not vary at 2 and 6 months, GLP-1 and GIP significantly increased at 6 months in both groups. In particular, GLP-1 increased more rapidly in obese participants, while the increase of GIP followed similar trends across visits in both groups.

*Conclusions.*    Nonsurgical treatment of periodontitis is associated with increased GLP-1 and GIP levels in non-obese and obese patients; changes in GLP-1 were more rapid in obese participants. This might have positive implications for the metabolic risk of these individuals.

**Key words:** periodontitis; obesity; incretin axis

**Introduction**

Periodontitis is a polymicrobial chronic inflammatory disorder characterised by a broad spectrum of systemic implications, including an increased cardiovascular risk (1,2). It is caused by a dysbiosis of the oral microbiota and often coexists with several metabolic diseases; diabetes, obesity and metabolic syndrome (3-5). It has been suggested that, second to smoking, obesity is amongst the strongest putative risk factors for inflammatory periodontal tissue destruction. Epidemiological surveys, such as the National Health and Nutrition Examination Survey (NHANES III), have reported correlations between measures of adiposity (BMI, waist circumference, skinfold thickness) and severity of periodontitis (6). Important associations between central adiposity and tooth loss have been reported also in non-obese populations (7). Chronic, low grade inflammation is considered the potential mechanism linking periodontitis and obesity; the excess of adipose tissue leads to an excessive secretion of hormones and proinflammatory cytokines that may sustain the systemic inflammatory processes, aggravate gingival inflammation, facilitate bacterial proliferation on the tooth surfaces, ultimately promoting the development or progression of periodontitis, and compromising healing response to periodontal therapy (8, 9). Insulin resistance, a hallmark of obesity, may also play a role, being an independent predictor of gingival/periodontal inflammation over time in overweight/obese individuals (10).

Obesity is characterised by a dysregulation of incretins secretion and actions. Individuals with obesity, but otherwise healthy, display a reduced GLP-1 response to luminal nutrients, and blocking or attenuating GIP activity might facilitate a correction of lifestyle-induced obesity. In recent years, GLP-1 analogues have been proposed as a successful therapeutic strategy aimed at losing weight in obese individuals, even in the absence of type 2 diabetes (11-13). Incretin axis is strongly influenced also by gut microbiota (14, 15), and a powerful

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effect of periodontal treatment on oral microbiota composition has been reported, sometimes with relevant clinical implications (16, 17); on the other hand, therapeutic manipulations of microbiota by administration of probiotics has been hypothesised to promote oral health (18). We recently described, for the first time, a metabolically unfavourable panel of incretins (high glucagon and GIP and reduced GLP-1 levels) in obese patients with periodontitis (19). The present study addresses whether intensive periodontal treatment might correct these abnormalities and investigates the role of oral and systemic inflammation on the potential changes induced by treatment. Therefore the aim of this analysis was to investigate the potential influence of an intensive periodontal treatment on the association between periodontal inflammation and GIP, and GLP-1 levels in obese and non-obese individuals.

**Participants and Methods**

*Participants*    58 obese (BMI $\geq$ 30 kg/m$^2$) and 57 non obese (BMI <25 kg/m$^2$) individuals, without diabetes, referred to the UCLH Eastman Dental Hospital (Periodontology Unit), participated in the study. Inclusion criteria were a minimum age of 35 years, non-smoker, with a confirmed diagnosis of moderate-to-severe periodontitis (probing pocket depths of $\geq$5mm and marginal alveolar bone loss with >30% sites affected), and at least 15 teeth present.

Exclusion criteria were presence of diabetes, uncontrolled systemic diseases (cardio-vascular diseases including hypertension, liver diseases, pulmonary diseases, end-stage renal failure, or neoplasm), hepatitis B or HIV infection, chronic treatment (>2 weeks) with drugs known to affect periodontal tissues (phenytoin or cyclosporin), chronic systemic antibiotic treatment, and pregnancy or lactation. The group of non-obese individuals with periodontitis and without diabetes served as controls. The diagnosis of type 2 diabetes was excluded based on

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the patient self-reported clinical history and the absence of treatment with glucose-lowering medications (20).

*Study procedures*      At baseline, patients attended the clinical research centre after at least 6 hours of fasting to undergo clinical periodontal examination and a blood sample collection. Clinical parameters recorded included medication use, tobacco exposure (current, former, or never smoker), blood pressure, height, body weight, waist circumference and anthropometric measures. After collection, blood samples were immediately processed, obtaining serum and plasma aliquots. Serum was analysed for glucose, total cholesterol, triglycerides, HDL cholesterol, creatinine; estimated glomerular filtration rate (eGFR) was calculated by the CKD-EPI formula. Plasma was kept at -70°C for subsequent measurement of hormones and markers of inflammation and oxidative stress.

Clinical periodontal status was assessed by a single trained examiner and consisted of full-mouth probing pocket depth (PPD), recession of the gingival margin (REC) relative to the cementoenamel junction at six sites per tooth. The presence or absence of supragingival dental plaque and gingival bleeding on probing were also recorded. The average full-mouth number of periodontal lesions (probing depth, >4 mm), the score for full-mouth gingival bleeding on probing, and the score for full-mouth plaque were calculated for each patient, as previously described (21).

At a subsequent visit, essential patient oral hygiene self-care coaching was provided. All patients underwent intensive periodontal treatment, as previously described (20). Briefly, following administration of local anaesthesia, full mouth mechanical periodontal debridement (instrumentation to remove all soft and hard deposits from the tooth surface) was performed in a single session (2-3 hours). The same assessments and procedures performed at the baseline visit were repeated at 2 and 6 months following the treatment.

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*Biochemistry*  Concentrations of glucose and standard lipid fractions (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, creatinine) were measured using standard assays and an automated analyser (Cobas 8000 analyser, Roche, Mannheim, Germany). Serum high sensitivity C-reactive protein (hs-CRP) concentrations were determined by immunoturbidimetry (Cobas Integra 700, Roche, Mannheim, Germany). The levels of malondialdehyde (MDA) were also measured, given that this is a recognised marker of lipid peroxidation and, as such, a reliable measure of systemic oxidative stress, leading to cellular damage. MDA levels were assessed by thiobarbituric acid-reactive substances (TBARS), according to Wasowicz *et al.* (22), with modifications. Briefly, 100µl of plasma or standard solution was diluted in 1ml of distilled water, adding 1mL of TBA solution (29 mM 2-thiobarbituric acid in 8.75 M acetic acid adjusted to pH 2.4). The mixture was placed in a water bath heated for 1 h at 95°C, ice cooled and centrifuged at 1500x for 5 min at room temperature. Absorbance of supernatant was determined at 532 nm with a plate reader spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). The calibration curve was set up using 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, St. Louis, MO, USA) diluted in distilled water, to achieve concentrations of 100, 50, 25, 12.5, 6.25, 3.12,1.56,0.78,0.39 and 0 µM.

Insulin was measured by an electro-chemiluminescence assay on a COBAS e411 (Roche, Indianapolis, IN, USA). Plasma GLP-1, GIP, and glucagon were assessed using a Multiplex technique (Millipore Corp, Billerica, MA, USA and Mercodia AB, Uppsala, SW). HOMA Index was calculated according with the standard formula: (Insulin concentration * Glucose concentration) / 22.5 (23). Beta cell activity was estimated by applying the HOMA2 model (24).

*Statistics*      Data are reported as mean ± SD, unless otherwise specified. Clinical characteristics at baseline were compared by paired *t*-test for numerical variables and Fisher's exact test for categorical variables. Differences between baseline, 2 months and 6 months in parameters of periodontal health and concentrations of circulating biomarkers were analysed by repeated measures analysis of variance, considering the group (obese/non-obese) as between factor and visits as within factor. Given the substantial differences in some parameters at baseline potentially affecting the different incretin response to periodontal treatment between obese and non-obese groups, the analyses were repeated using a propensity score approach to minimise the potential effect of confounding. As previously described (20), the propensity score for each participant was created using a logistic regression model where the BMI status (obese/non-obese) was considered the outcome variable and factors potentially associated with obesity that may influence a different incretin response after treatment were taken as explanatory variables (age, gender, ethnicity, periodontal pocket depth -PPD -, and glucose levels). This propensity score was then included as a covariate in the models regressing BMI group (normal/obese) against the levels of each incretin at 2 months and 6 months. Spearman rank correlation was used to assess the presence of significant associations between variables. Because of the nature of this study as a post-hoc analysis, no formal power calculation was performed. All analyses were performed using SPSS ver21 and a two-sided p-value of less than 0.05 was regarded as significant.

**Results**

Obese and non-obese individuals were similar in terms of gender distribution but those recruited in the obese group were younger, less frequently of White European ancestry and had higher values of systolic and diastolic blood pressure throughout the study than the non-obese group, with no significant changes in the trends of blood pressure between groups (**Table 1**). Both groups maintained a stable BMI over the duration of the study. None was a current smoker. Renal function was similarly preserved in both groups (eGFR: $97.8\pm16.4$ ml/min/1.73m$^2$ in obese and $98.2\pm20.1$ ml/min/1.73m$^2$ in non-obese subjects).

At baseline, there were some differences between groups in the clinical parameters of periodontal status, especially in terms of severity of disease with higher mean PPD in obese than non-obese individuals. The treatment resulted in improvement in periodontal health in both groups, such that all parameters were significantly improved from baseline to 2 and 6 months post treatment. However, the obese group demonstrated a slightly poorer response to treatment than the non-obese (**Table 2**).

Obese participants were characterised by higher HOMA index and HOMA%B, fasting plasma glucose and triglycerides but lower HDL cholesterol at baseline. None of these parameters seemed to vary substantially after the periodontal treatment. Similarly, hs-CRP was higher in obese than in non-obese, but the effect of the treatment between the two groups was negligible. MDA levels were similar in obese and non-obese at baseline and were not influenced by periodontal treatment (**Table 3).**

At baseline, there were no significant associations between measures of periodontitis and circulating levels of glucoregulatory hormones, except for GLP-1 that was directly associated with periodontal pocket depth (r = 0.207 pmol/l change of GLP-1 per mm change in mean periodontal pocket depth (mm); p=0.026), mean clinical attachment levels (r = 0.186 pmol/l

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change of GLP-1 per mm change in mean clinical attachment levels; $P = 0.046$) and the full mouth bleeding score ($r = 0.195$ pmol/l change of GLP-1 per 1 unit change of the full mouth bleeding score; $p=0.037$). As expected, in the whole study group GLP-1 levels were related with HOMA%B ($r=0.340$, $p<0.001$).

Associations of treatment and the glucoregulatory hormone pattern of change were then evaluated. The incretin profile analysis over time in the study population revealed baseline insulin levels were higher in obese than in non-obese participants, being unaffected by periodontal treatment **(Figure 1);** accordingly, no significant relationship emerged between improvement in periodontal parameters and HOMA%B variation.

Although circulating levels of glucagon were significantly higher in the obese than the non-obese group at baseline ($p=0.006$), no significant visit-group interaction was observed, suggesting a non-significant difference between obese and non-obese participants in the changes of glucagon levels induced by periodontal treatment. Adjustment for age and ethnicity did not change the results. When the analysis was repeated using the propensity score, a significant difference in glucagon levels between obese and non-obese individuals was observed at 2 months after treatment ($p=0.005$), while at 6 months this difference was not statistically significant.

Quite surprisingly, at baseline, there was no significant difference in the levels of GLP-1 between obese and non-obese participants. The treatment was associated with a significant increase in the GLP-1 concentrations in both groups by the end of the study (+40% in obese and +67% in non-obese at 6 months, $p<0.001$ vs baseline visit); however, the changes in the GLP-1 levels induced by treatment followed a different kinetic between groups. Compared to the non-obese group, the participants with obesity presented with a more rapid increase in GLP-1 between baseline and 2 months (+3% vs 19%, respectively). Instead, the non-obese group presented a more rapid increase of GLP-1 between 2 and 6 months when compared to

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the obese group. Therefore, at 2 months circulating levels of GLP-1 were significantly higher in the obese compared to the control group (42 pmol/l vs 27 pmol/l, p<0.001). After adjustment for age and ethnicity, the visit-group interaction term remained significant (p=0.029). The analyses, repeated using the propensity score, confirmed the significant difference between obese and non-obese group in the levels of GLP-1 at 2 months (p<0.001) but not 6 months after treatment.

Lastly, levels of GIP did not differ at baseline and significantly changed across visits with a similar trend in both groups (Figure 1). The greatest and significant increase in GIP levels was observed between the 2-month and 6-month visit (p<0.001 in both groups). These changes were not affected by adjustments for ethnicity and age, neither related to HOMA%B. The use of propensity score confirmed the lack of a significant difference in the GIP circulating levels at 2 and 6 months from treatment between the obese and non-obese group.

**Discussion**

The main results of the present study are to show, for the first time, that periodontitis treatment could improve the pattern of glucoregulatory hormones in obese and non-obese individuals; such improvement mainly comes through an increase of GLP-1 and GIP, and furthermore, is evident over a different time frame in obese vs non-obese individuals. The novelty of these preliminary findings is that the glucoregulatory hormone changes occurred without relevant alterations of the metabolic phenotype of the patients studied; no weight reduction, or variations in lipid profile or blood pressure levels were reported. Another important and novel observation is that, in the presence of periodontitis, GLP-1 levels of non-obese and obese individuals are similar, suggesting a role for the oral inflammation even more relevant than the obesity status in influencing incretin axis.

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Adults with obesity have a higher probability of periodontal diseases (25); we recently reported that obesity might also impair the healing of periodontal tissues after non-surgical periodontal treatment, in agreement with preliminary studies (20, 26, 27) . Obese participants, presenting on average with more severe measures of periodontitis at baseline, experience some level of clinical response to periodontal treatments, with an improvement of the various indices when observed in comparison with the similar changes in non-obese, suggesting that obesity might be associated with a more severe presentation or evolution of the periodontitis (28).

The lack of variations in circulating markers of subclinical inflammation is in keeping with previous reports describing the long-term, systemic impact of an intensive periodontal treatment in patients with moderate-to-severe periodontitis (29, 30). As this study did not include a control/no treatment group, it was not possible to compare the effect of intensive treatment on markers of inflammation.  The low basal TBARS levels, similar to those of controls, also suggest that biomarkers of systemic oxidative stress might not be the ideal candidates to exactly mirror degree and seriousness of oral involvement. The scope of this study analysis, however, was not to observe the effects of an acute inflammatory response on the regulation of the glucose metabolism, rather to assess the long-term impact of a potential resolution of the oral disease after intensive treatment on the incretin axis. Indeed, this study design did not include a blood collection 24 hours and 1 week after periodontal treatment, that are known to be associated with the development of an acute inflammatory response (29, 31).

The effect of periodontal treatment on glucoregulatory hormones deserves attention. We have recently shown that patients with obesity and periodontitis display an impaired level of GLP-1 and GIP and a relative hyperglucagonemia, likely participating in their impaired glucose tolerance (19); the present study shows a different impact of periodontal treatment on such

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hormones. In fact, while insulin is able to predict the severity of periodontal involvement in obese individuals (10), its circulating levels seem not to change after periodontal treatment in both groups, similarly to what was described in the previous reports where no changes in glucose or insulin levels were recorded in obese participants without diabetes undergoing full-mouth periodontal debridement (32). Glucagon levels, lower than those reported in our previous study (conducted in more severely obese individuals), were unaffected by periodontal treatment. In interpreting these results, however, it should be acknowledged that given the very low levels of glucagon at baseline and its large variation in the included population, the result of this analysis is likely to be underpowered, therefore further studies should investigate to confirm these findings.

Surprisingly, basal GLP-1 levels did not differ between non-obese and obese participants affected by periodontitis, suggesting a prevailing effect of the oral disease over the obesity state. GLP-1 and GIP could play an intriguing role in the metabolic dysregulation of patients with periodontitis, irrespective of the presence of diabetes; in fact, GIP promotes bone formation (33), and periodontal bacteria contribute to degradation of human incretins (34). In this context, hypoglycaemic agents characterised by an anti-inflammatory activity, or by a protective effect on bone tissue, might be helpful in counteracting the progression of periodontitis (35). . This hypothesis is particularly relevant for obese individuals, since high doses of these compounds are now being indicated for the treatment of obesity, irrespective of the presence of type 2 diabetes (13, 36). Therefore, the increased GLP-1 and GIP levels observed in this study population after the periodontal treatment, might be regarded as a novel, relevant real-life evidence supporting experimental observations of a favourable interplay between oral microbiota and incretin axis, which is not mediated by changes in systemic inflammation, and more likely linked to the direct mouth-gut cross-talk as anatomical and functional continuum. In fact, at baseline GLP-1 levels were similar in non-

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obese and obese, correlate with the severity of periodontal inflammation, and increase after the periodontal treatment. Overall, these data suggest that the potential impact of periodontal treatment on microbiota composition and subsequent incretin variations outweigh the role of adiposity. Intriguingly, the time-course of such response differs between non-obese and obese participants, being more rapid in the latter. When considering the lack of change in insulin resistance or β cell activity (as from the HOMA Index and HOMA%B), it could be hypothesised that obese individuals might rapidly and positively respond, in metabolic terms, to an adequate treatment of periodontitis. Indeed, in the presence of periodontitis, the main pathogen, i.e. *Porphyromonas gingivalis,* may migrate to the colon, proliferate, and modify gut functions (37, 38). Our data, by providing a novel clinical, real-life support for the existence of such complex connection between gut hormones and periodontitis in obesity, an axis the importance of which is frequently underestimated, further advocates the potential use of GLP-1 receptor agonists when managing these patients. Further research should be performed on this topic.

We would also point out as we put effort in performing an adequate clinical phenotyping of the recruited population, for example by measuring serum creatinine and calculating glomerular filtration rate, given the well described relationship between periodontal status and renal impairment (39), and relevant prognostic implications of such relationship (40).

It is acknowledged that there are some limitations in this analysis. The most relevant is the lack of post-meal serum samples to better explore the incretin axis in the post-prandial phase, when the clinical relevance of such hormone pattern is maximised. We also acknowledge the relatively small population sample investigated, and the possibility of undiagnosed type 2 diabetes. However, mean fasting glucose values were pretty far from the diagnostic threshold in both obese and nonobese subjects, and none of the participants had never registered a fasting glucose value suggestive for type 2 diabetes. Lastly, the inherent limitation of this

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being a secondary analysis of a non-randomised study without a no treatment control group cannot be overlooked. Hence, we urge caution in interpreting the evidence presented and suggest it to be considered in support of a novel hypothesis to be tested in the future.

In conclusion, this study showed for the first time that periodontal treatment might positively influence gut hormones in patients with mild-to-moderate obesity, likely contributing to the metabolic improvement exerted by such treatments in patients at high risk to progress toward type 2 diabetes and/or to develop cardiovascular disease. Data on larger cohorts, either coming from clinical studies or from real-world evidence, are merited to confirm these observations, together with studies specifically designed to explore the effects of incretins on periodontitis.

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**Data availability**    The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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**Figure Legends**

**Figure 1.** Changes in circulating levels of gluco-regulatory hormones in the obese and non-obese groups following periodontal therapy. GIP was log-transformed because of a non-normal distribution.

¥ = Significant difference between baseline and 6 months; ‡ = Significant difference between 2 months and 6 months; * = Significant difference between obese and lean group.

**Table 1.** Demographic characteristics at baseline, 2 and 6 months following treatment

| Variable | Group | Baseline | 2 Months | 6 Months |
|---|---|---|---|---|
| Age (years) | Non-obese | 50±8 | | |
| | Obese | 47±7 ¥ | | |
| Gender (male, %) | Non-obese | 50 | | |
| | Obese | 40 | | |
| Current smoking habits (%) | Non-obese | 0 | | |
| | Obese | 0 | | |
| Ethnicity (White European, %) | Non-obese | 71 | | |
| | Obese | 51 ¶ | | |
| Systolic Blood Pressure (mmHg) | Non-obese | 120±14 | 119±14 | 119±12 |
| | Obese | 131±17 † | 128±12 † | 128±14 † |
| Diastolic Blood Pressure (mmHg) | Non-obese | 76±10 | 74±10 | 75±8 |
| | Obese | 86±12 † | 83±9† | 83±9† |
| BMI (Kg/m$^2$) | Non-obese | 23.10±1.34 | 23.07±1.41 | 23.14±1.57 |
| | Obese | 35.58±5.42 † | 35.54±5.37 † | 35.53±5.56 † |

† P < 0.001 for the comparison with the non-obese group at the same visit.

¶ P < 0.01 for the comparison with the non-obese group at the same visit.

¥ P < 0.05 for the comparison with the non-obese group at the same visit.

**Table 2**     Periodontal characteristics at baseline, 2 and 6 months following treatment

| Variable | Group | Baseline | 2 Months | 6 Months |
|---|---|---|---|---|
| Mean Periodontal Pocket Depth (mm) | Non-obese | 3.37±0.62 | 2.55±0.45 * | 2.55±0.41 * |
|  | Obese | 3.69±0.72 § | 2.85±0.50 ¶* | 2.86±0.55 ¶* |
| Mean Clinical Attachment Level (mm) | Non-obese | 3.91±0.86 | 3.26±0.87 * | 3.26±0.85 * |
|  | Obese | 4.16±0.99 | 3.50±0.87 * | 3.46±0.88 * |
| Full mouth bleeding score (%) | Non-obese | 47.34±20.10 | 21.19±9.65 * | 21.73±10.14 * |
|  | Obese | 52.61±19.47 | 27.39±10.66 ¶* | 31.21±11.82 †* |
| Full mouth plaque score (%) | Non-obese | 61.52±19.04 | 25.80±16.09 * | 29.62±18.20 * |
|  | Obese | 64.14±17.89 | 30.59±17.96 * | 32.67±20.86 * |

† $P < 0.001$ for the comparison with the non-obese group at the same visit.

¶ $P < 0.01$ for the comparison with the non-obese group at the same visit.

§ $P < 0.05$ for the comparison with the non-obese group at the same visit.

* $P < 0.05$ compared to baseline for the within group comparison.

The presence of a significant difference was assessed using analysis of variance for repeated measures between obese and normal weight groups. When the model indicated a significant change of the assessed parameter across visits, pairwise comparison with the use of paired T-test were performed.

**Table3**   Blood sample results at baseline, 2 and 6 months following treatment

| Variable | Group | Baseline | 2 Months | 6 Months |
|---|---|---|---|---|
| Tot Cholesterol (mmol/L) | Non-obese | 5.11±0.97 | 5.09±0.88 | 5.11±0.90 |
| | Obese | 5.34±1.16 | 5.24±1.13 | 5.21±1.11 |
| LDL Cholesterol (mmol/L) | Non-obese | 3.14±0.89 | 3.09±0.81 | 3.08±0.83 |
| | Obese | 3.45±0.98 | 3.38±0.95 | 3.36±0.95 |
| HDL Cholesterol (mmol/L) | Non-obese | 1.56±0.36 | 1.60±0.37 | 1.60±0.37 |
| | Obese | 1.22±0.33 † | 1.23±0.30 † | 1.24±0.32 † |
| Triglycerides (mmol/L) | Non-obese | 0.88±0.39 | 0.87±0.33 | 0.92±0.39 |
| | Obese | 1.46±0.84 † | 1.40±0.77 † | 1.36±0.66 † |
| Glucose (mmol/L) | Non-obese | 4.98±0.48 | 4.91±0.40 | 4.82±0.46 |
| | Obese | 5.28±1.52 | 5.45±2.32 | 5.32±1.07 ¶ |
| Insulin (mU/L) | Non-obese | 6.52±4.35 | 6.43±3.24 | 6.33±3.44 |
| | Obese | 14.44±7.58 † | 14.35±5.74 † | 15.01±8.81 † |
| HOMA Index* | Non-obese | 1.23 [0.82-1.82] | 1.29 [0.87-1.83] | 1.20 [0.94-1.71] |
| | Obese | 3.00 [2.20-3.96] † | 3.05 [2.16-4.11] † | 2.88 [2.24-4.69] † |
| HOMA%B* | Non-obese | 77.95 [66.20-97.40] | 83.40 [69.30-101.50] | 84.40 [68.05-102.10] |
| | Obese | 137.90 [109.20-162.20] † | 141.60 [108.90-167.60] † | 132.00 [103.10-157.70] † |
| hs-CRP (mg/L)* | Non-obese | 0.60 [0.40-1.20] | 0.60 [0.40-1.10] | 0.70 [0.40-1.50] |
| | Obese | 3.70 [1.50-7.30] † | 3.15 [1.30-6.20] † | 3.05 [1.10-5.70] † |
| MDA (μmol/L)* | Non-obese | 2.15±0.50 | 2.05±0.41 | 2.31±1.31 |
| | Obese | 2.32±0.63 | 2.41±0.73 | 2.51±1.02 |

† P < 0.001 for the comparison with the non-obese group at the same visit.

¶ P < 0.01 for the comparison with the non-obese group at the same visit.

No significant longitudinal changes were observed in the clinical characteristics (within subject, across visits)

* Not normally distributed variables

**Figure 1**

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