

Effects of Improving Glycemic Control with Insulin on Leptin, Adiponectin, Ghrelin and Neuropeptide Y Levels in Patients with Type 2 Diabetes Mellitus: a Pilot Study

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Abstract: *Objective:* Insulin therapy is associated with weight gain in patients with type 2 diabetes mellitus (T2DM). Several peptides are implicated in appetite control. We evaluated the effects of insulin-induced improved glycaemic control on leptin, adiponectin, ghrelin, neuropeptide Y (NPY) levels and patient characteristics.

Method: Consecutive T2DM patients (n = 90) were divided into 2 groups: Group A: 45 insulin-naïve uncontrolled (glycosylated haemoglobin A_{1c}; HbA_{1c} >7%) patients on oral hypoglycaemic agents (OHAs) who converted to insulin monotherapy. Group B: 45 well-controlled (HbA_{1c} <7%) patients on OHAs. Both groups were monitored at baseline, 3 and 6 months. Males and females were analyzed separately because some hormone levels differ between genders.

Results: In both genders, insulin therapy (Group A) was associated with significant (p = 0.003 to <0.001) increases in weight, body mass index and leptin levels and significant decreases in glucose, HbA_{1c} and NPY levels. In male insulin-treated patients a significant increase in adiponectin levels (p = 0.008) was observed. There were significant correlations (p = 0.016 to <0.001) between leptin levels, waist circumference and body fat in all patient groups, except group B males.

Conclusion: Changes in leptin, adiponectin and NPY levels may occur after insulin-induced improved glycaemic control. These changes may be influenced by gender, weight, body fat and HbA_{1c}.

Keywords: Gender, glycosylated haemoglobin A_{1c}, insulin, type 2 diabetes mellitus, weight gain.

INTRODUCTION

In patients with type 2 diabetes mellitus (T2DM) tight glycaemic control with either intensive insulin therapy or sulfonylureas has been associated with weight gain [1, 2]. However, insulin-treated patients gained more weight compared with those on oral hypoglycaemic agents (OHAs) [2].

The underlying mechanisms causing insulin-induced weight gain are only partly understood and include (apart from the correction of dehydration in some cases) the anabolic effects of insulin, stimulation of lipogenesis, suppression of hepatic glucose production, reduction of basal metabolic rate and elimination of glycosuria [1, 3, 4]. Furthermore, fear of hypoglycaemia and a feeling of freedom to eat in

patients on insulin therapy may account for additional food consumption [3, 5]. On the other hand, a central effect of insulin has also been proposed with insulin and leptin resistance leading to reduced satiety signaling to the hypothalamus despite overfeeding and hyperinsulinaemia [3, 6].

Nutrients and gastrointestinal hormones like ghrelin are considered as short-term signals that influence food intake [7]. In contrast, insulin and leptin act as long-term regulators that ensure stability of body weight and adiposity and maintenance of energy homeostasis over a more prolonged period of time [7]. These regulators transfer information from the periphery to the central nervous system, where they modulate neuropeptide release which in turn influences appetite and energy expenditure [8]. In particular, leptin stimulates the expression of anorexigenic neuropeptides and inhibits the expression of orexigenic neuropeptides, such as neuropeptide Y (NPY), therefore suppressing food intake and enhancing energy expenditure [8]. Both central and peripheral adiponectin administration reduced body weight *via* either food intake suppression or increased energy expenditure [9].

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Ghrelin increases NPY expression in the hypothalamus and blocks leptin-induced satiety, subsequently leading to increased food intake [10].

In this pilot study we evaluated the effect of insulin-induced improvement of glycaemic control on adipokines (i.e. leptin and adiponectin), a gut peptide (i.e. ghrelin) and a neuropeptide (i.e. NPY) levels, weight parameters, lipids and renal function in patients with T2DM. We also considered the possible correlations between these variables.

MATERIALS AND METHODS

Study Population

A total of 90 consecutive patients with T2DM were recruited from the Diabetes Outpatient Clinic, AHEPA University Hospital, Thessaloniki, Greece. The study population was divided into 2 groups: Group A consisted of 45 insulin-naïve poorly controlled (glycosylated haemoglobin A_{1c}; HbA_{1c} >7%) patients on OHAs who were converted to insulin monotherapy (2 daily injections of biphasic isophane insulin). Group B consisted of 45 well-controlled (HbA_{1c} <7%) patients on OHAs. All patients in group B were taking metformin (800 mg, twice daily) and 30 of them were also taking sulfonylureas, mainly gliclazide (80 mg, 2 or 3 times daily). Patients with history of impaired liver or kidney function, malignancy, endocrinopathy or on corticosteroid treatment were excluded. All women were postmenopausal and were not on hormone replacement therapy.

Patients on thiazolidinediones were excluded as these agents may influence leptin (rosiglitazone) [11], adiponectin (rosiglitazone [12] and pioglitazone [13]) and ghrelin levels (rosiglitazone) [14].

Study Design

Both groups were prospectively followed up for 6 months and monitored on an outpatient basis at baseline, 3 and 6 months. In the insulin-treated group, dose adjustments were based on home blood glucose monitoring. At every evaluation, physical examination including weight, height, waist circumference (WC) and blood pressure measurements, determination of percentage of body fat by bioimpedance analysis (SKYLARK BT-905, Taiwan) and laboratory analyses (white cell count (WCC), glucose, HbA_{1c}, creatinine (Cr), uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (gamma-GT), creatine kinase (CK), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), leptin, adiponectin, ghrelin and NPY) were performed in all patients.

Body mass index (BMI = weight/height²), estimated glomerular filtration rate (eGFR) based on the Modification of Diet in Renal Disease (MDRD) formula (http://nephron.org/mdrd_gfr_si) and creatinine clearance based on the Cockcroft-Gault formula (<http://clinicalcalculator.com/english/nephrology/cockcroft/ca.htm>) were also calculated.

At baseline, patients were interviewed by a qualified dietician. A specific 7-day diet sheet was given to each patient

at every visit. Daily calorie intake was 500 calories below the needs of the patient, taking into account the individual's physical activity. The patients were advised to maintain the same diet and level of physical activity throughout the study.

The study was approved by the local ethics committee and informed consent was obtained from all participants.

Blood Sample Collection and Analysis

All blood samples were collected in the morning after an overnight fast (water allowed) and divided into 2 samples of 10 ml each. The first 10 ml were immediately analyzed for routine laboratory parameters (WCC, glucose, HbA_{1c}, Cr, uric acid, ALT, AST, gamma-GT, CK, TC, TG, HDL-C and LDL-C). The second 10 ml, after centrifugation at 4°C, were stored in aliquots at -20°C until assayed for leptin, total adiponectin, total ghrelin and NPY using commercially available radioimmunoassay kits (Linco Research Inc., St. Charles, MO, USA for leptin, adiponectin and ghrelin; Euro-Diagnostica Inc., Malmö, Sweden for NPY). It is known that ghrelin circulates in 2 forms: octanoylated (acylated) and desacylated. Although only acylated ghrelin is bioactive, levels of total ghrelin are a good surrogate for those of acylated ghrelin because the ratio of the 2 remains constant under a wide variety of conditions [15, 16].

Regarding intra- and inter-assay variations, for leptin, intra-assay variation is 8.3, 4.6, 3.9, 4.7 and 3.4% CV for 5 varying concentrations of leptin, i.e. 4.9, 7.2, 10.4, 15.7 and 25.6 ng/ml, respectively, whereas inter-assay variation is 6.2, 5.0, 4.7, 3.0 and 3.6% CV 4.9, 7.2, 10.4, 15.7 and 25.6 ng/ml, respectively.

For adiponectin, intra-assay precision is 3.59, 6.21 and 1.78% CV for low (3 ng/ml), middle (6 ng/ml) and high (15 ng/ml) concentration of adiponectin, respectively, whereas inter-assay precision is 9.25, 6.9 and 9.25% CV for low, middle and high adiponectin levels, respectively.

For total ghrelin, intra-assay variation is 10.0, 3.3, 7.9 and 4.4% CV for 4 varying concentrations of ghrelin, i.e. 1000, 1500, 2000 and 3000 pg/ml, respectively, whereas inter-assay variation is 14.7, 17.8, 16.0 and 16.7% CV for 1000, 1500, 2000 and 3000 pg/ml, respectively.

For NPY, intra-assay precision is 3.9 and 2.6% CV for 57.9 and 100 pmol/L respectively, whereas inter-assay precision is 12.7 and 10.5% CV for 59.1 and 98.9 pmol/L, respectively.

Statistical Analysis

Statistical analyses were performed using the SPSS version 13.0 software package (SPSS Inc., Chicago, USA). Values are expressed either as mean ± SD for parametric variables or as median (range) for non parametric ones. Differences between groups at baseline, 3 and 6 months were analyzed by Student's t test, Mann Whitney and χ^2 test for parametric, non parametric continuous and categorical variables, respectively. Repeated measures ANOVA or the non-parametric alternative Friedman test was used to compare related samples data throughout the study. Correlations between the 4 peptides and various parameters were deter-

mined by Pearson r or Spearman Rho correlation analyses. Due to multiple comparisons, significance was set at p (2-tailed) ≤ 0.025 [17].

Males and females were considered separately because leptin and adiponectin levels differ significantly between genders [18,19]. Furthermore, patients were recruited sequentially and therefore we could not ensure equal gender distribution in both groups. In order to verify our results we also assessed the correlations between glucose and HbA_{1c} [20], as well as between body fat and WC [21].

RESULTS

Results at Baseline

Male Patients (Table 1)

Groups A and B were similar for hypertension, statin treatment, cardiovascular disease (CVD), smoking, age, weight, height, BMI, WC, duration of diabetes, systolic blood pressure (SBP), diastolic blood pressure (DBP), WCC, uric acid, HDL-C, LDL-C, AST, ALT, CK, Cr, leptin, adiponectin, ghrelin, NPY, MDRD eGFR and Cockcroft-Gault creatinine clearance. However, in group B body fat was significantly higher ($p = 0.005$), whereas TC ($p = 0.005$), TG ($p = 0.012$) and gamma-GT ($p = 0.012$) were significantly lower compared with group A.

Female Patients (Table 2)

Groups A and B were similar for hypertension, statin treatment, CVD, smoking, age, weight, height, WC, duration of diabetes, SBP, WCC, uric acid, TG, HDL-C, LDL-C, AST, ALT, CK, gamma-GT, Cr, leptin, adiponectin, MDRD eGFR and Cockcroft-Gault creatinine clearance. However, in group B BMI ($p = 0.009$) and body fat ($p < 0.001$) were significantly higher, whereas DBP ($p = 0.013$), TC ($p = 0.022$), ghrelin ($p = 0.019$) and NPY ($p = 0.021$) were significantly lower compared with group A.

For both genders we did not compare glucose and HbA_{1c} values at baseline since we defined group A as uncontrolled and group B as well-controlled patients with T2DM.

Results at 3 Months

Male Patients (Table 3)

NPY levels were higher in group B compared with group A ($p = 0.017$).

Female Patients (Table 4)

Body fat was higher ($p = 0.004$) and HDL-C levels were lower ($p = 0.001$) in group B compared with group A.

Results at 6 Months

Male Patients (Table 5)

Fasting glucose ($p = 0.005$) and leptin levels were lower ($p = 0.003$) in group B compared with group A.

Female Patients (Table 6)

Ghrelin levels were lower ($p < 0.001$) and NPY levels were higher ($p = 0.01$) in group B compared with group A.

Changes Throughout the Study in Groups A and B

Male Patients

In group A weight ($p < 0.001$), BMI ($p < 0.001$), leptin ($p < 0.001$) and adiponectin levels ($p = 0.008$) increased significantly, whereas fasting glucose ($p < 0.001$), HbA_{1c} ($p < 0.001$) and NPY levels ($p < 0.001$) decreased significantly. No differences were observed in group B male patients.

Female Patients

In group A weight ($p = 0.001$), BMI ($p < 0.001$), HDL-C ($p = 0.002$) and leptin levels ($p = 0.003$) increased significantly, whereas fasting glucose ($p < 0.001$), HbA_{1c} ($p < 0.001$), DBP ($p = 0.003$), TG ($p = 0.006$) and NPY levels ($p < 0.001$) decreased significantly. In group B female patients, body fat ($p = 0.002$), AST ($p = 0.012$) and ghrelin levels ($p < 0.001$) were significantly reduced.

Correlations

Leptin levels significantly correlated with WC and body fat in all patient groups, except group B male patients (Table 7).

As expected, glucose correlated significantly with HbA_{1c} in all groups and at all times ($p = 0.019$ to < 0.0001), except for group A females at baseline and 3 months. Furthermore, body fat correlated significantly with WC in all groups and at all times ($p = 0.01$ to < 0.0001), except group B male patients (Table 7).

DISCUSSION

Leptin levels were higher in females compared with males in each group, at all sampling points during the study ($p \leq 0.001$ for all comparisons). These findings are consistent with previous results in T2DM patients on various hypoglycaemic treatments [18]. Adiponectin concentrations were non-significantly higher in females in each group, at baseline and at 6 months. Circulating adiponectin levels are reported to be higher in females with or without DM in some but not all studies [19, 22, 23]. No significant gender differences in ghrelin concentrations were found in each group at all sampling points during the study. This is in agreement with reports in nondiabetic individuals [24], although higher circulating ghrelin concentrations in nondiabetic females have been documented [25]; data in T2DM patients are lacking. NPY levels did not differ significantly between genders in each group at all times during the study. No literature regarding gender differences for NPY were found.

Some of the differences we observed between groups at baseline may be attributed to the uncontrolled patients being more concerned about their lifestyle (e.g. weight). Uncontrolled group A females had significantly higher baseline ghrelin levels compared with well-controlled group B female patients ($p = 0.019$). In contrast, others reported that patients with T2DM had lower ghrelin levels when long-term glycaemic control was poor [26]. The observed lower baseline ghrelin concentrations in group B females may be attributed to their higher BMI and body fat compared with group A females, as ghrelin levels are negatively correlated with both

Table 1. Baseline Clinical and Fasting Laboratory Characteristics of Male Patients with type 2 Diabetes Mellitus

| | Group A (n=26) | Group B (n=15) | p |
|---|----------------|----------------|-------|
| Hypertension (%) | 57.7 | 73.3 | ns |
| Statin use (%) | 42.3 | 53.3 | ns |
| CVD (%) | 42.3 | 20 | ns |
| Smoking (%) | 38.5 | 20 | ns |
| Age (years) | 66.9±8.1 | 68.1±6.8 | ns |
| Weight (kg) | 74.6±14 | 77.4±9.5 | ns |
| Height (m) | 1.7±0.1 | 1.7±0.1 | ns |
| BMI (kg/m ²) | 26.4±4.1 | 27.6±3.2 | ns |
| Waist circumference (cm) | 97.9±12.8 | 97.3±7.8 | ns |
| Body fat (%) | 31.8±9.6 | 41.3±10 | 0.005 |
| Duration of diabetes (years) | 12.8±7.5 | 10.9±9.5 | ns |
| SBP (mmHg) | 131.5±22 | 136.3±18.1 | ns |
| DBP (mmHg) | 78.5±15.2 | 76.3±11.1 | ns |
| WCC (x10 ³ /μl) | 7319±1805 | 6311±1896 | ns |
| Uric acid (mg/dl) | 5.3±1.5 | 5.6±1.2 | ns |
| TC (mg/dl) | 193±36 | 157±37 | 0.005 |
| TG (mg/dl) | 172±91 | 109±34 | 0.012 |
| HDL-C (mg/dl) | 47±12 | 45±10 | ns |
| LDL-C (mg/dl) | 112±32 | 90±30 | ns |
| AST (u/l) | 20±10 | 22±12 | ns |
| ALT (u/l) | 22±12 | 45±81 | ns |
| CK (u/l) | 69±39 | 135±159 | ns |
| Gamma-GT (u/l) | 31 (12-138) | 19 (8-304) | 0.012 |
| Glucose (mg/dl) | 216±79 | 131±25 | N/A |
| HbA _{1c} (%) | 9.8±1.7 | 6±0.9 | N/A |
| Cr (mg/dl) | 1±0.3 | 0.9±0.2 | ns |
| Leptin (ng/ml) | 7.6±7.4 | 4.3±2.3 | ns |
| Adiponectin (μg/ml) | 11.5±8.4 | 15.6±9.7 | ns |
| Ghrelin (pg/ml) | 874±292 | 810±176 | ns |
| NPY (pmol/L) | 156.5±57.8 | 127.4±29.5 | ns |
| MDRD eGFR (mL/min/1.73m ²) | 84.5±22.8 | 96.6±22.4 | ns |
| Cockcroft-Gault creatinine clearance (mL/min) | 79.9±26.2 | 91.6±22.6 | ns |

CVD: cardiovascular disease (coronary heart disease and/or stroke); BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; WCC: white cell count; TC: total cholesterol; TG: triglyceride; HDL-C: high density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; AST: aspartate transaminase; ALT: alanine transaminase; CK: creatine kinase; gamma-GT: gamma glutamyl transpeptidase; Cr: creatinine; NPY: neuropeptide Y; MDRD: modification of diet in renal disease; eGFR: estimated glomerular filtration rate; N/A: not applicable.

Table 2. Baseline Clinical and Fasting Laboratory Characteristics of Female Patients with Type 2 Diabetes Mellitus

| | Group A (n=19) | Group B (n=30) | p |
|---|-----------------|-----------------|--------|
| Hypertension (%) | 63.2 | 83.3 | ns |
| Statin use (%) | 52.6 | 56.7 | ns |
| CVD (%) | 21.1 | 16.7 | ns |
| Smoking (%) | 31.6 | 13.3 | ns |
| Age (years) | 61.8±11.4 | 64.5±6.6 | ns |
| Weight (kg) | 69.1±19.4 | 75.3±14.2 | ns |
| Height (m) | 1.6±0.1 | 1.6±0.1 | ns |
| BMI (kg/m ²) | 27.1±7.2 | 31±4.9 | 0.009 |
| Waist circumference (cm) | 96.6±16.8 | 105.8±13 | ns |
| Body fat (%) | 41±7.9 | 50.7±7.9 | <0.001 |
| Duration of diabetes (years) | 11.3±6 | 9.7±6.8 | ns |
| SBP (mmHg) | 141.1±19.8 | 136.3±16.6 | ns |
| DBP (mmHg) | 85.8±13.1 | 75.7±11.4 | 0.013 |
| WCC (x10 ³ /μl) | 7799±3277 | 7132±1551 | ns |
| Uric acid (mg/dl) | 4.5±1.6 | 5.5±1.4 | ns |
| TC (mg/dl) | 222±45 | 192±41 | 0.022 |
| TG (mg/dl) | 160 (69-620) | 129 (69-542) | ns |
| HDL-C (mg/dl) | 58±18 | 49±12 | ns |
| LDL-C (mg/dl) | 125±41 | 113±33 | ns |
| AST (u/l) | 20±12 | 21±6 | ns |
| ALT (u/l) | 24±17 | 24±15 | ns |
| CK (u/l) | 60±27 | 82±36 | ns |
| Gamma-GT (u/l) | 17 (12-120) | 15 (10-53) | ns |
| Glucose (mg/dl) | 232±59 | 136±35 | N/A |
| HbA _{1c} (%) | 10.3±1.7 | 6.2±0.7 | N/A |
| Cr (mg/dl) | 0.8±0.3 | 0.8±0.2 | ns |
| Leptin (ng/ml) | 14.3±8.8 | 17.8±8.1 | ns |
| Adiponectin (μg/ml) | 15.9±8.4 | 16.8±11.1 | ns |
| Ghrelin (pg/ml) | 934±245 | 809±314 | 0.019 |
| NPY (pmol/L) | 186.1±71.9 | 140.9±45.7 | 0.021 |
| MDRD eGFR (mL/min/1.73m ²) | 82.8±30.9 | 88.6±28.2 | ns |
| Cockcroft-Gault creatinine clearance (mL/min) | 87.2±54.5 | 95.8±38.4 | ns |

For abbreviations see Table 1.

BMI and body fat in T2DM patients [27]. However, ghrelin levels remained significantly higher in group A females compared with group B females ($p < 0.001$) 6 months after

initiation of insulin therapy, even though BMI and body fat became similar between these groups.

Table 3. Fasting Laboratory and Other Characteristics of Male Patients with Type 2 Diabetes Mellitus at 3 Months

| | Group A (n=26) | Group B (n=15) | p |
|---|----------------|----------------|-------|
| Weight (kg) | 76.1±14.4 | 77.5±9.4 | ns |
| BMI (kg/m ²) | 26.9±4.2 | 27.7±3.3 | ns |
| Waist circumference (cm) | 96.7±12.4 | 98.1±8.3 | ns |
| Body fat (%) | 34.5±13 | 37.5±6.8 | ns |
| SBP (mmHg) | 137.7±18.2 | 133.7±12.9 | ns |
| DBP(mmHg) | 76.9±12.1 | 76.7±10.5 | ns |
| WCC (x10 ³ /μl) | 6782±2174 | 6953±1472 | ns |
| Uric acid (mg/dl) | 5.7±1.5 | 5.7±0.9 | ns |
| TC (mg/dl) | 190±49 | 173±28 | ns |
| TG (mg/dl) | 129±58 | 136±53 | ns |
| HDL-C (mg/dl) | 48±10 | 48±11 | ns |
| LDL-C (mg/dl) | 116±40 | 97±23 | ns |
| AST (u/l) | 19±5 | 21±9 | ns |
| ALT(u/l) | 19±7 | 23±11 | ns |
| CK (u/l) | 106±60 | 99±86 | ns |
| Gamma-GT (u/l) | 30 (10-75) | 17 (10-475) | ns |
| Glucose (mg/dl) | 151±51 | 123±31 | ns |
| HbA _{1c} (%) | 7±1.6 | 5.8±0.9 | N/A |
| Cr (mg/dl) | 0.9±0.3 | 0.9±0.2 | ns |
| Leptin (ng/ml) | 10.4±7.7 | 6.7±4.3 | ns |
| Adiponectin (μg/ml) | 15±11.6 | 11.7±7.3 | ns |
| Ghrelin (pg/ml) | 933±574 | 801±227 | ns |
| NPY (pmol/L) | 111.7±26.3 | 133.4±27.6 | 0.017 |
| MDRD eGFR (mL/min/1.73m ²) | 87.1±24.7 | 95.5±27.1 | ns |
| Cockcroft-Gault creatinine clearance (mL/min) | 84.7±32.9 | 90.8±25.5 | ns |

For abbreviations see Table 1.

Group A males had higher leptin levels than group B males at 6 months. This agrees with previous reports of higher leptin concentrations in T2DM patients on insulin therapy compared with those on other hypoglycaemic treatment [18].

Group A females had significantly higher HDL-C concentrations compared with group B females at 3 months ($p = 0.001$). This difference may be attributed to the insulin-induced improvement of HbA_{1c} observed at 3 months, as good glycaemic control is associated with increased HDL-C levels in T2DM patients [28]. However, no similar results were observed in our male patients.

In the present study, after 6 months of insulin therapy, weight, BMI and leptin levels significantly increased,

whereas fasting glucose, HbA_{1c} and NPY levels significantly decreased in both males and females. These insulin-associated changes have been reported [29-32], although without considering genders separately.

Aas *et al.*, suggested that, since the increase in leptin concentrations precedes weight gain during insulin therapy, insulin per se and not increased fat mass is responsible for the observed changes [30]. The sustained rise in body weight despite the significant simultaneous increase in leptin levels may be explained by leptin resistance in diabetic patients [32].

Adiponectin levels significantly increased only in insulin-treated males. Similarly, Miyashita *et al.*, [33] reported that adiponectin concentrations increased in patients with

Table 4. Fasting Laboratory and Other Characteristics of Female Patients with type 2 Diabetes Mellitus at 3 Months

| | Group A (n=19) | Group B (n=30) | p |
|---|----------------|----------------|-------|
| Weight (kg) | 72.6±19.9 | 75.3±13.9 | ns |
| BMI (kg/m ²) | 28.5±7.4 | 31.1±4.9 | ns |
| Waist circumference (cm) | 100.9±17.2 | 104.9±12.1 | ns |
| Body fat (%) | 39.2±8.6 | 46.3±6.2 | 0.004 |
| SBP (mmHg) | 136.3±22.1 | 181.8±270.3 | ns |
| DBP(mmHg) | 75.5±13.8 | 77.3±12 | ns |
| WCC (x10 ³ /μl) | 6840±1693 | 7387±1515 | ns |
| Uric acid (mg/dl) | 4.9±1.3 | 5.6±1.3 | ns |
| TC (mg/dl) | 196±40 | 196±42 | ns |
| TG (mg/dl) | 125±57 | 168±94 | ns |
| HDL-C (mg/dl) | 65±18 | 50±12 | 0.001 |
| LDL-C (mg/dl) | 106±34 | 113±30 | ns |
| AST (u/l) | 23±10 | 20±6 | ns |
| ALT(u/l) | 25±15 | 22±9 | ns |
| CK (u/l) | 83±34 | 97±73 | ns |
| Gamma-GT (u/l) | 15 (7-292) | 16 (10-79) | ns |
| Glucose (mg/dl) | 141±50 | 141±34 | ns |
| HbA _{1c} (%) | 7.2±1.4 | 6.2±0.8 | N/A |
| Cr (mg/dl) | 0.8±0.2 | 0.8±0.2 | ns |
| Leptin (ng/ml) | 23.4±13.5 | 20.2±10.9 | ns |
| Adiponectin (μg/ml) | 12.9±11.5 | 11.3±5.3 | ns |
| Ghrelin (pg/ml) | 907±284 | 836±315 | ns |
| NPY (pmol/L) | 118.9±22.9 | 137.4±43.8 | ns |
| MDRD eGFR (mL/min/1.73m ²) | 88.3±25.9 | 87.9±31.4 | ns |
| Cockcroft-Gault creatinine clearance (mL/min) | 95.7±48.3 | 94.1±36.2 | ns |

For abbreviations see Table 1.

T2DM after initiation of insulin treatment (twice-daily biphasic insulin analogue). In contrast, Jacob *et al.*, reported a non significant trend for a reduction in adiponectin levels in T2DM patients on insulin monotherapy or insulin plus metformin [29]. These discrepancies could be explained by differences in sample size and HbA_{1c} values achieved. Furthermore, neither Miyashita *et al.*, [33] nor Jacob *et al.*, [29] analyzed their results according to gender.

Ghrelin levels did not change significantly in the insulin monotherapy groups, as previously reported [31]. In contrast, Jacob *et al.*, documented significant decreases in ghrelin concentrations after initiation of insulin therapy in T2DM patients [29].

It has been demonstrated that NPY is expressed and secreted by adipose tissue and that peripherally administered

insulin stimulates the release of adipogenic NPY [34]. As NPY has been reported to exert antilipolytic properties, hyperinsulinemia may lead to an increase in adiposity *via* elevated circulating NPY levels [34]. These interactions may partly explain the hyperinsulinemia-related weight gain. In contrast, in the present study, NPY levels significantly decreased in both males and females following the initiation of insulin therapy. Leptin has been reported to suppress the hypothalamic expression of NPY [35]; thus increased leptin levels may lead to reductions in NPY concentrations in both the hypothalamus and the circulation. It should be noted that we do not know if circulating NPY levels represent changes in the hypothalamus. There is evidence that NPY can cross the blood brain barrier [36] but this has only been demonstrated in animals. The fall in NPY levels observed in our study may reflect a complex interaction of several factors

Table 5. Fasting Laboratory and Other Characteristics of Male Patients with Type 2 Diabetes Mellitus at 6 Months

| | Group A (n=26) | Group B (n=15) | p |
|---|----------------|----------------|-------|
| Weight (kg) | 78.2±14.6 | 77.4±9.6 | ns |
| BMI (kg/m ²) | 27.7±4.3 | 27.6±3.4 | ns |
| Waist circumference (cm) | 100.5±12.1 | 97.1±9.1 | ns |
| Body Fat (%) | 34.6±10.1 | 35.9±8 | ns |
| SBP (mmHg) | 136.4±18.5 | 129.3±16.1 | ns |
| DBP (mmHg) | 78.9±12.8 | 73±5.9 | ns |
| WCC (x10 ³ /μl) | 7425±1906 | 6269±1389 | ns |
| Uric acid (mg/dl) | 5.5±1.7 | 5.8±0.9 | ns |
| TC (mg/dl) | 188±61 | 171±32 | ns |
| TG (mg/dl) | 136±58 | 130±76 | ns |
| HDL-C (mg/dl) | 45±10 | 47±8 | ns |
| LDL-C (mg/dl) | 108±33 | 97±23 | ns |
| AST (u/l) | 21±6 | 25±9 | ns |
| ALT (u/l) | 20±8 | 26±9 | ns |
| CK (u/l) | 114±53 | 128±150 | ns |
| Gamma-GT (u/l) | 26 (10-200) | 18 (8-494) | ns |
| Glucose (mg/dl) | 158±52 | 119±19 | 0.005 |
| HbA _{1c} (%) | 6.8±1.5 | 5.7±0.8 | N/A |
| Cr (mg/dl) | 1±0.3 | 0.9±0.2 | ns |
| Leptin (ng/ml) | 10.6±7.3 | 4.5±2.6 | 0.003 |
| Adiponectin (μg/ml) | 17.5±9.2 | 18.7±18.2 | ns |
| Ghrelin (pg/ml) | 818±255 | 662±285 | ns |
| NPY (pmol/L) | 85.8±30.9 | 108±36.7 | ns |
| MDRD eGFR (mL/min/1.73m ²) | 83.5±20.4 | 93.8±27 | ns |
| Cockcroft-Gault creatinine clearance (mL/min) | 82.8±25.5 | 89.7±25.6 | ns |

For abbreviations see Table 1.

(e.g. leptin resistance, obesity and stress). Further research is needed to clarify the role of peripheral and central NPY levels on appetite control.

Apart from appetite, NPY has been associated with intense or prolonged stress, leading to vasoconstriction and angiogenesis [37]. Interestingly, plasma NPY concentrations were reported to be increased due to enhanced sympathetic activation in stress-related cardiac conditions [38].

Therefore, an increase in circulating NPY levels cannot be attributed separately to changes in nutritional or stress status.

Of note, in patients on hemodialysis, plasma NPY concentrations were elevated due to both a poor state of nourishment and fluid volume overload-induced stress [39].

Body fat has been proposed as the best predictor of leptin levels in humans independently of gender [40]. In the present study a non significant increase in the percentage of body fat was observed following insulin therapy, as reported by others [29]. Interestingly, a significant insulin-related increase in both subcutaneous fat area and fat mass was also observed in T2DM patients [29, 30]. These discrepancies may be attributed to differences in methodology (e.g. definition of body fat).

In our study, leptin levels correlated with body fat (%) and WC in all groups, except group B males. Similarly, WC correlated with leptin concentrations in Asian-Indian patients with impaired glucose tolerance or T2DM [41, 42]. In the present study, changes in leptin levels did not correlate with HbA_{1c}, as reported by Aas *et al.*, [30].

Table 6. Fasting Laboratory and Other Characteristics of Female Patients with Type 2 Diabetes Mellitus at 6 Months

| | Group A (n=19) | Group B (n=30) | p |
|---|----------------|----------------|--------|
| Weight (kg) | 73.3±21.2 | 76.2±14.4 | ns |
| BMI (kg/m ²) | 28.8±7.9 | 31.4±5.1 | ns |
| Waist circumference (cm) | 101±18.1 | 105.7±12.8 | ns |
| Body Fat (%) | 41.4±13.3 | 47.5±7.4 | ns |
| SBP (mmHg) | 139.7±24.6 | 139.5±11.7 | ns |
| DBP (mmHg) | 79.2±12.6 | 78.6±8.3 | ns |
| WCC (x10 ³ /μl) | 7186±1859 | 7418±1671 | ns |
| Uric acid (mg/dl) | 4.8±1.4 | 5.4±1.4 | ns |
| TC (mg/dl) | 198±35 | 191±34 | ns |
| TG (mg/dl) | 145±79 | 161±71 | ns |
| HDL-C (mg/dl) | 60±19 | 52±13 | ns |
| LDL-C (mg/dl) | 109±35 | 115±54 | ns |
| AST (u/l) | 22±9 | 17.2±6.9 | ns |
| ALT(u/l) | 23±11 | 22±9 | ns |
| CK (u/l) | 103±71 | 87±53 | ns |
| Gamma-GT (u/l) | 19 (9-299) | 16 (7-56) | ns |
| Glucose (mg/dl) | 142±55 | 139±35 | ns |
| HbA _{1c} (%) | 6.9±1.5 | 6.3±1.1 | ns |
| Cr (mg/dl) | 0.8±0.2 | 0.8±0.2 | ns |
| Leptin (ng/ml) | 23.3±12.8 | 17.4±7.9 | ns |
| Adiponectin (μg/ml) | 18.3±14.1 | 24.9±19.7 | ns |
| Ghrelin (pg/ml) | 874±346 | 542±312 | <0.001 |
| NPY (pmol/L) | 96.9±31.1 | 131.1±56.9 | 0.01 |
| MDRD eGFR (mL/min/1.73m ²) | 87.6±25.4 | 83±25.5 | ns |
| Cockcroft-Gault creatinine clearance (mL/min) | 96.4±49.7 | 91.4±34.8 | ns |

For abbreviations see Table 1.

Patients on OHAs did not show any change in weight or leptin levels throughout the study, as previously reported [30]. Group B females were the only patients to show significant reduction in both ghrelin levels and body fat. These results may be because they had the highest body fat (50.7 ± 7.9%) at baseline and the greatest reduction (33%) in ghrelin levels throughout the study compared with the other groups.

Throughout the study, TG levels significantly decreased ($p = 0.006$) and HDL-C levels significantly increased ($p = 0.002$) only in group A females. These results may be attributed to the insulin-induced improvement of HbA_{1c}, as good glycaemic control exerts beneficial effects on lipids in T2DM patients [28].

DBP significantly decreased in group A females 6 months after initiation of insulin therapy ($p = 0.003$). In this

context, others [43] reported a significant positive association of mean HbA_{1c} and DBP in patients with type 1 diabetes mellitus (T1DM); no data were found for T2DM patients.

Renal function indices did not change significantly throughout the study in all groups. However, all our patients had normal kidney function. In contrast, abnormal baseline GFR values in patients with T1DM improved significantly following intensive insulin treatment [44]; no data were found for T2DM patients.

Several drugs frequently prescribed to diabetic patients have been shown to influence leptin, adiponectin and/or ghrelin levels [45]. In detail, OHAs have been reported to differentially influence plasma levels of leptin, adiponectin and ghrelin [11-14, 46-48], whereas there were no reports for NPY. Antihypertensive agents were shown to either increase

Table 7. Correlations Between Waist Circumference, Body Fat and Leptin Levels Observed Throughout the Study

| Male Patients | | | | Female Patients | | | |
|---------------|-------------------|-------------------------|---------|-------------------------|---------|-------------------------|---------|
| Group A | | | | Group A | | Group B | |
| | | Correlation Coefficient | p | Correlation Coefficient | p | Correlation Coefficient | p |
| Baseline | Leptin - WC | rho = 0.812 | < 0.001 | rho = 0.653 | 0.002 | r = 0.61 | < 0.001 |
| | Leptin - body fat | rho = 0.664 | < 0.001 | rho = 0.72 | 0.001 | r = 0.566 | 0.001 |
| | WC - body fat | r = 0.721 | < 0.001 | r = 0.839 | < 0.001 | r = 0.533 | 0.003 |
| | | Correlation Coefficient | p | Correlation Coefficient | p | Correlation Coefficient | p |
| 3 months | Leptin - WC | rho = 0.764 | < 0.001 | rho = 0.725 | 0.001 | r = 0.606 | < 0.001 |
| | Leptin - body fat | rho = 0.668 | < 0.001 | rho = 0.772 | < 0.001 | r = 0.538 | 0.002 |
| | WC - body fat | r = 0.725 | < 0.001 | r = 0.799 | < 0.001 | r = 0.512 | 0.004 |
| | | Correlation Coefficient | p | Correlation Coefficient | p | Correlation Coefficient | p |
| 6 months | Leptin - WC | rho = 0.686 | < 0.001 | r = 0.747 | < 0.001 | r = 0.6 | 0.003 |
| | Leptin - body fat | rho = 0.469 | 0.016 | r = 0.641 | 0.003 | r = 0.641 | 0.003 |
| | WC - body fat | r = 0.632 | 0.01 | r = 0.752 | < 0.001 | r = 0.587 | 0.001 |

WC: waist circumference.

[49] or reduce leptin [50] and to increase adiponectin levels in T2DM patients [51, 52]. No data were found regarding the influence of these agents on ghrelin and NPY levels in T2DM patients. In these patients lipid-lowering drugs were also reported to reduce leptin [53, 54] and increase adiponectin concentrations [12, 55]. In contrast, ghrelin levels did not change following short-term (6 weeks) treatment with either atorvastatin or fenofibrate in diabetic patients with mixed hyperlipoproteinaemia [56]. There are no data concerning the effects of lipid-modifying agents on NPY concentrations.

In our study no patient was on fibrate therapy but almost half of them were taking statins and even more patients were on antihypertensive agents. The study groups did not differ significantly in statin and antihypertensive use and these treatments remained unchanged throughout the study. Nevertheless, the use of different drugs in our patients is a limitation of our study as is the small sample size which did not allow multivariate analysis. However, other studies in this field [29-33] had similar patient numbers and did not analyze their results by gender. Furthermore, the measured parameters are interrelated and the influence of each individual factor on insulin-related body weight gain is difficult to identify.

CONCLUSIONS

In the present study, initiation of insulin treatment was associated with a significant increase in leptin levels and a significant decrease in NPY levels in both genders. These effects should have suppressed appetite and reduced food consumption, leading to weight loss. In contrast, all insulin-treated patients gained weight, possibly due to other mechanisms such as the anabolic effects of insulin, stimulation of lipogenesis, suppression of hepatic glucose production, re-

duction of basal metabolic rate and elimination of glycosuria. It is also possible that leptin resistance may be partly responsible for the observed weight increase.

In general, changes in leptin, adiponectin and NPY levels may occur following insulin-induced improved glycaemic control. These changes may be influenced by several variables, including gender, weight, body fat and glycaemic control. The interactions between these factors were not considered in previous studies.

It is possible that targeting the peptides measured in this study could influence weight gain after insulin treatment.

DECLARATION OF INTEREST

This paper was written independently. The authors did not receive financial or professional help with the preparation of the manuscript.

DPM has given talks, attended conferences and participated in advisory boards and trials sponsored by various pharmaceutical companies (MSD, Genzyme, Astra-Zeneca).

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