Association of insulin and cholesterol levels with peripheral nervous system function in overweight adults: A 3-year follow-up

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Abstract

Purpose: The purpose of this prospective 3-year follow-up was to investigate the association of glucose, insulin and cholesterol levels with peripheral nervous system (PNS) function in overweight and obese subjects.

Methods: Forty non-diabetic overweight and obese adults were enrolled, of whom twenty-nine completed the follow-up. PNS function was measured and defined by conduction studies of the peroneal motor nerve and the radial, sural, and medial plantar sensory nerves. Serum insulin and glucose levels were determined with an oral glucose tolerance test (OGTT), and cholesterol levels were measured. The measurements were performed at baseline and after 3 years.

Results: The change in serum insulin level at 120 min after an OGTT was positively associated with changes in peroneal nerve conduction velocity (NCV) and F-wave mean, sural NCV, and medial plantar NCV. Action potential amplitudes decreased consistently and significantly in all sensory nerves.

Conclusion: The change in serum insulin level at 120 min appears to be positively associated with changes in nerve conduction velocity over 3 years but not with nerve action potential amplitudes. Significant decreases in the action potential amplitudes of all sensory nerves suggest that such changes might be the earliest detectable sign of damage to the PNS in overweight and obese people without type 2 diabetes.

Introduction

Overweight is a major global burden that causes high morbidity and mortality and predisposes individuals to diseases such as type 2 diabetes, metabolic syndrome and hypertension. Diabetes is a well-known factor causing axonal damage and segmental demyelination to the peripheral nervous system (PNS) (1). This disease is called diabetic neuropathy, and it is one of the most common complications of diabetes. Its symptoms usually include numbness, tingling sensations, pain and weakness.

Recently, there have been several discoveries indicating that damage to the PNS may occur even in the earlier stages of diabetes spectrum. Furthermore, abnormal glucose tolerance is associated with metabolic syndrome, which also includes hypertension, hyperlipidemia and obesity, and these factors may also be independent risk factors for peripheral neuropathy. An association between impaired glucose tolerance (IGT) and peripheral neuropathy has been reported (2-4). Elevated triglyceride levels have been associated with the progression of diabetic neuropathy (5). Metabolic syndrome and waist circumference have been associated with increased risk of peripheral neuropathy (6, 7). Overweight individuals also seem to have lower motor and sensory nerve action potential amplitudes compared with normal-weight subjects (8, 9).

In our previous cross-sectional study, we demonstrated a positive association between insulin levels and nerve conduction velocities in overweight and obese people (10), even though insulin resistance is a well-known pre-stage of impaired fasting glucose and impaired glucose tolerance possibly leading ultimately to type 2 diabetes. The aim of this prospective 3-year follow-up was to investigate the

association between glucose, insulin and cholesterol levels and PNS function in overweight and obese subjects.

Materials and Methods

Forty overweight and obese adults (mean age 49 years, SD 11 years, range 28 - 68 years) were recruited from an outpatient clinic. The inclusion criterion was a BMI ≥ 25. Subjects with type 2 diabetes were excluded from the study. Height was measured without shoes to the nearest 0.5 cm. Weight was measured on a calibrated scale to the nearest 0.1 kg. All study subjects provided informed consent, and the study was approved by the local ethics committee.

Fasting, 30-min and 120-min glucose and insulin levels were measured with a standardized 75 g oral glucose tolerance test. Serum glucose levels were determined using a hexokinase assay (Konelab analyzers, Thermo Electron Oy, Vantaa, Finland), and insulin levels were determined using a chemiluminescent microparticle immunoassay (Abbot Diagnostic, Abbot Park, IL, USA). Insulin resistance was assessed with the quantitative insulin sensitivity check index (QUICKI). This method is significantly correlated with the hyperinsulinemic euglycemic glucose clamp and is considered a valid method for evaluating insulin resistance in obese patients (11). The QUICKI was calculated with the following equation:

$$QUICKI = 1 / (\log \text{fasting insulin}) \times (\log \text{fasting plasma glucose})$$

Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride analyses were performed using an autoanalyzer (ADVIA 1650 Chemistry System, Siemens Medical Solutions, Tarrytown, USA), and low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation (12).

The conduction of the peroneal motor nerve and the radial, sural, and medial plantar sensory nerves, with standard distance, were measured bilaterally using Keypoint 4 and Keypoint portable devices (Medtronic, Skövlunde, Denmark). Using surface electrodes, sensory nerve conduction was measured antidromically for the radial and sural nerves and orthodromically for the medial plantar nerves. For the motor nerve conduction measurements, the distal and proximal latency, distal and proximal peak-to-peak compound muscle action potential (CMAP), nerve conduction velocity (NCV), F-wave minimum, mean and maximum latency and number of F-waves per 20 stimuli (persistence) were determined. For the sensory nerve conduction measurements, the onset latency, peak-to-peak sensory nerve action potential (SNAP) amplitude, and NCV were determined. The skin surface temperature was measured immediately following the conduction measurements. The filter settings were 2 Hz to 10 kHz for the motor nerve conduction measurements and 20 Hz to 2 kHz for the sensory nerve conduction measurements. The follow-up measurements were done by the same investigators.

Statistical analysis

Mean values and standard deviations were used as the descriptive statistics. Serum triglyceride and insulin levels were normalized by a logarithmic transformation. Univariate associations between the explanatory and response variables were analyzed using Pearson’s correlation coefficients. Student's t-test was used for the statistical comparisons (unpaired for the drop-out analysis and paired for the follow-up analysis). All variables found to be significant in the univariate analyses were entered into the multivariate analyses. A multiple stepwise linear regression analysis was used to assess the relationship between changes in the insulin levels, cholesterol levels, weight and nerve conduction

measurements. Closely interrelated variables were entered separately in these models. All models were adjusted for skin temperature changes. The Bonferroni method was used to adjust P values for multiple comparisons. Otherwise, $p < 0.05$ was considered statistically significant. The SAS 9.1 (SAS Institute Inc. Cary, NC, USA) software program was used for all statistical analyses.

Results

Eleven individuals (9 females and 2 males) failed to participate in either the nerve conduction studies or the oral glucose tolerance tests in the follow-up. The drop-outs differed from those who completed the study regarding insulin levels at 120 min (78.4 µU/ml vs. 44.5 µU/ml, $p = 0.03$) and sural SNAP amplitude (27.1 µV vs. 21.4 µV, $p = 0.004$).

Table 1 shows the characteristics of the remaining 29 study participants (17 females and 12 males) and a comparison between baseline and the 3-year follow-up measurements**.** The mean age of the individuals at the end of the follow-up was 52 (SD 11) years. In the beginning of the study, 6 participants were overweight (BMI 25-30) and 23 were obese (BMI > 30). At the end of the follow-up 4 were overweight and 25 were obese. Two individuals had impaired fasting glucose and two had impaired glucose tolerance in the beginning of the study according to WHO criteria. At the end of the follow-up, four individuals had impaired fasting glucose, and four individuals had impaired glucose tolerance; no one had developed diabetes. At the end of the follow-up, there was a slight increment in weight (NS) and waist circumference ($p = 0.04$), along with the levels of fasting insulin ($p = 0.01$) and glucose (NS). Total cholesterol ($p = 0.002$), LDL cholesterol ($p = 0.003$) and QUICKI ($p = 0.02$) decreased significantly. Serum insulin level at 120 min (NS) and glucose level at 120 min (NS) slightly decreased.

The neurophysiological data of the study participants are presented in Table 2. All sensory nerve action potential amplitudes decreased significantly ($p < 0.001$) during the follow-up. Significant changes in the peroneal motor nerve variables were seen, namely, increases in distal CMAP amplitude ($p = 0.02$), proximal CMAP amplitude ($p = 0.01$) and F-wave min ($p = 0.01$) and decreases in distal latency ($p = 0.03$) and F-wave persistence ($p = 0.03$). Furthermore, the medial plantar latency increased ($p = 0.02$). There were no statistically significant changes in nerve conduction velocities during the 3-year follow-up.

Correlation analysis revealed that individual level change in insulin level at 120 in was significantly related with individual level changes in peroneal NCV, medial plantar NCV and near significantly with sural NCV (Table 3). Furthermore, to reveal the possible predictors of change in PNS function over time at the individual level, we performed a multivariable stepwise regression analysis adjusting for skin temperature change and adding changes in the serum 120-min glucose and insulin levels, weight and HDL cholesterol and triglyceride levels to the models. The change in insulin level at 120 min after an OGTT was significantly and positively associated with changes in the peroneal NCV and F-wave mean, sural NCV, and medial plantar NCV with coefficients of determination of 15 %, 22 %, 10 % and 25 %, respectively. HDL cholesterol level change was positively associated with the change in the radial SNAP amplitude and triglyceride level change was significantly and inversely associated with the change in the sural SNAP amplitude with coefficients of determination of 15 % and 16 %, respectively. However, after Bonferroni correction, only 120-min serum insulin level change was found to be an independent predictor of medial plantar NCV change.

Discussion

In this 3-year follow-up study, we investigated the relationship of glucose, insulin and cholesterol levels to PNS function in 29 overweight adults. The change over 3 years in serum insulin level at 120 min after an OGTT seems to be positively related with the changes in nerve conduction velocities. Sensory nerve action potential amplitudes decreased significantly over time without being significantly associated with changes in glucose, insulin or cholesterol levels. The decrease in all sensory nerve action potential amplitudes suggests that such change might be the earliest detectable sign of damage to PNS in overweight and obese people without type 2 diabetes.

In this study, a positive relationship between the serum insulin level at 120 min after an OGTT and nerve conduction velocities was found. Elevated insulin levels due to obesity might have at least a temporary compensatory effect on PNS function. However, the sensory nerve action potential amplitudes decreased consistently and significantly during the 3-year follow-up. None of the study participants developed type 2 diabetes during the follow-up, and only a few had impaired glucose tolerance or impaired fasting glucose. This suggests that the earliest detectable negative alterations in PNS function in overweight and obese people without hyperglycemia are possibly seen in SNAP amplitudes.

Miscio et al. (9) found faster conduction velocities in peroneal motor nerve and ulnar and sural sensory nerves in obese subjects compared to normal-weight controls. Landau et al. (13) presented a similar finding, with BMI correlating positively with elbow ulnar motor nerve conduction velocities. It has been suggested that this phenomenon might be due to thermal insulation by thicker subcutaneous fat maintaining a higher temperature around the nerve (8). Our results contribute to the speculation on the phenomenon by suggesting that elevated insulin levels due to obesity might be one

of the biological factors explaining why obese people without hyperglycemia might demonstrate faster nerve conduction velocities in some nerves.

Previous studies have shown that insulin is a neurotrophic factor responsible for regulating neuronal growth, survival and differentiation (14, 15). This might explain our finding that the change over 3 years in serum insulin level at 120 min after an OGTT seems to be positively related to the changes in nerve conduction velocities. The positive relationship found in this study suggests that insulin might have a direct effect on myelination of the peripheral nerves. A recent study suggests that in the presence of hyperinsulinemia, the peripheral nervous system can also become insulin resistant and, therefore, be unable to respond to the neurotrophic properties of insulin (16). Considering the important role of insulin as a neurotrophic factor, it was recently suggested that dysfunction in insulin signaling might result in neurodegeneration and, thus, might be an important factor in the pathogenesis of diabetic neuropathy (17). In this study, QUICKI values decreased significantly, suggesting that insulin resistance increased among our study subjects.

Grote et al. (Grote et al. 2013a) demonstrated that both DRG and peripheral nerve respond to intrathecal insulin. Interestingly, in another study (Grote et al. 2013b) they further demonstrated that only peripheral nerve is responsive to low doses of systemic insulin, suggesting that DRG and peripheral nerve have different insulin signaling properties. Furthermore they also demonstrated that muscle, liver, and adipose tissue are more sensitive to insulin than PNS and may be acting as a “sink” and therefore reduce the available insulin to the PNS and therefore reduce the neurotrophic response. These recent findings may explain why we only found positive association between insulin level at 120 min and nerve conduction velocity and not with amplitudes. It might be that the more insulin is produced systemically due to insulin resistance the more insulin is available for the peripheral nerves to be utilized.

In this study, noteworthy negative alterations in neurophysiological variables were observed only in the sensory nerve action potential amplitudes, most dominantly in the sural and medial plantar sensory nerves. Supporting the findings in these distal nerves, a previous study suggested that in addition to the routinely used sural sensory nerve, the medial plantar sensory nerve is also a sensitive indicator of early damage in diabetic neuropathy because of its distal location (18). It has been shown that obese people tend to have significantly lower action potential amplitudes compared to normal-weight controls, which might be due to the dampening effect of a thicker subcutaneous fat layer (8, 9). In our study, sensory nerve action potential amplitudes decreased consistently and significantly even though our study subjects did not have significant weight gain. This suggests that in addition to the dampening effect of the thicker subcutaneous fat, there might be biological factors decreasing nerve action potential amplitudes in overweight and obese people without hyperglycemia. Based on this study, it is not possible to state whether there was actual loss of sensory axon fibers or whether there could have been formation of conduction blocks blocking the signal and thus decreasing the SNAP amplitudes.

We found that increments in triglyceride level change reduced the sural SNAP amplitude change and that increments in HDL cholesterol level change increased the radial SNAP change. However, after Bonferroni correction, these associations were no longer statistically significant. Pittenger et al. (19) found a negative correlation between HDL cholesterol and sural SNAP amplitude, and they concluded that there might be a sensory neuropathy cosegregating with features of metabolic syndrome. Smith and Singleton (20) demonstrated that obesity and triglycerides were related to loss of small unmyelinated axons. These findings indicate that cholesterol levels might have a direct association with PNS function. We could not find any variable significantly explaining the reduction

of SNAP amplitudes. It might be due to the complex nature of peripheral nerve pathogenesis; hence, our results are most likely explained by a combination of multiple factors such as hyperlipidemia, insulin resistance in the PNS and oxidative stress.

This study has several limitations. Our ability to draw definitive conclusions was limited by the relatively small sample size. The number of the drop-outs was quite high. Our explanatory variables for glucose metabolism were limited to only the insulin and glucose levels. Our PNS function measurement was limited to nerve conduction study, which does not reveal the status of small fibers. Furthermore, nerve conduction study does not clearly reveal whether the damage to the PNS has been axonal or demyelinating. QUICKI values might not be a representative evaluation of possible insulin resistance in the PNS.

Conclusion

We conclude that the change over 3 years in serum insulin level at 120 min after an OGTT seems to be positively related to the changes in nerve conduction velocities. Significant decreases in sensory nerve action potential amplitudes indicate that in overweight people without type 2 diabetes, it might be the earliest sign of negative alterations in PNS function. Further studies investigating the possible roles of insulin, insulin resistance and hyperlipidemia in the pathogenesis of prediabetic neuropathy are necessary.

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Table 3. Correlation between the individual level change in serum insulin level at 120 min after an OGTT and the individual level change in nerve conduction velocities over 3-years (n=29)

Variable	Δ Peroneal NCV	Δ Radial NCV	Δ Sural NCV	Δ Medial Plantar NCV
Δ Insulin level at 120 min	0.44 (0.02)*	0.16 (0.40)	0.36 (0.05)	0.57 (0.001)

Δ = change from baseline; * = correlation coefficient (p-value)