CLINICAL STUDY

The nicotinic acid analogue acipimox increases plasma leptin and decreases free fatty acids in Type 2 diabetic patients

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Abstract

The effect of 3 days of intensive treatment with acipimox, an antilipolytic nicotinic acid derivative, on plasma leptin levels was studied in eight patients with Type 2 diabetes mellitus in a double-blind, placebo-controlled, cross-over study. Acipimox reduced plasma free fatty acids (FFA) markedly and lowered plasma triglycerides, glucose and insulin. Plasma leptin levels were elevated in all eight patients during 3 days of acipimox treatment (mean increase $\pm$ S.E.: 2.38 ± 0.57 ng/ml, $P < 0.005$) and the 24 h mean effect of acipimox on leptin levels increased during the experimental period ($P < 0.03$). The effect on plasma insulin and glucose resembled a mirror image of the effect on plasma leptin during 3 days of treatment. The suggestion that leptin mediates insulin resistance and may be involved in the development of the diabetic syndrome cannot be supported by the present results. It has been reported that FFA stimulates leptin secretion. Surprisingly, despite a markedly reduced FFA level, leptin concentration increased in the present study. We suggest that a primary acipimox effect is to increase leptin secretion, and that this prevails over the reduced FFA stimulus.

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Introduction

Patients with Type 2 diabetes mellitus have increased plasma free fatty acids (FFA) (1) and this increase is positively correlated with glycaemia (2, 3). In normal subjects ingestion of FFA lowers insulin-stimulated glucose uptake (4, 5). Moreover, obese subjects have increased levels of FFA and are insulin resistant (6, 7). Thus, insulin resistance may be associated with increased FFA concentrations.

Studies of the effect of long-term administration of acipimox (5-methyl-pyrazine carboxylic acid 4-oxide), an antilipolytic nicotinic acid derivative, on FFA levels have given controversial results (8–11). Short-term administration of acipimox for up to 3 days, however, leads to a pronounced reduction of plasma FFA levels in Type 2 diabetic patients (9, 12–15). The decrease in plasma FFA is associated with a fall in plasma glucose, improved glucose tolerance and increased insulin-stimulated glucose uptake in muscle (9, 12–15).

Leptin is a hormone produced in adipocytes (16) and generally changes in leptin concentration are taken to reflect changes in secretion. The hypothalamus is the target for the satiety effects of leptin (17, 18) but leptin

also has peripheral effects in a number of tissues. It has been suggested that leptin directs metabolic fuels towards utilization and away from storage (19). Leptin increases energy expenditure in adipose tissue and skeletal muscle by increasing mitochondrial uncoupling proteins (20–23), which are important for thermogenesis from glucose and FFA (24–26).

It has been proposed in several studies that leptin mediates changes in insulin sensitivity. Some studies suggest that leptin causes peripheral insulin resistance. In cultured adipocytes, fibroblasts and hepatic cells it has been shown that leptin inhibits insulin-stimulated glucose uptake and phosphorylation of insulin receptor substrate-1 (27–29). It has, however, also been reported that leptin improves glucose uptake in skeletal muscle, the quantitatively most important tissue of glucose uptake (30). Sinha *et al.* (31) and Malstrøm *et al.* (32) found no independent effect of Type 2 diabetes *per se* on leptin concentrations. Furthermore, the development of insulin resistance has been described in certain patients in spite of a pronounced decrease in plasma leptin concentration (33). From the studies mentioned, the overall effect of leptin on insulin sensitivity is unclear.

A possible direct effect of acipimox on leptin secretion has never been addressed. Leptin is produced in adipose tissue, where short-term administration of acipimox inhibits the production of FFA (9, 12–15). The decrease in FFA is associated with improved glucose tolerance as previously described (9, 12–15). It is generally accepted that FFA stimulates leptin production (34, 35). We wanted to study whether the reduced FFA concentration during acipimox treatment had an impact on leptin secretion in Type 2 diabetic patients under conditions where insulin, another stimulator of leptin secretion (32, 36–39), was also reduced. Surprisingly, an increase in leptin concentration during acipimox treatment was found, probably as a result of a not previously described primary drug effect.

Subjects and methods

Subjects

Eight insulin-treated Type 2 diabetic patients were included in a previous study in which the effect of 3 days of intensified acipimox treatment on plasma FFA, triglycerides, glucose and insulin were studied (14). The Type 2 diabetes diagnosis was based on the age of onset of diabetes, duration of diabetes until insulin treatment was initiated, and C-peptide values before and after glucagon stimulation (14). Of the patients four were males and four females and their age was 58.9 ± 7.9 years (mean $\pm$ S.D.). Body mass index (BMI) is shown for each patient in Fig. 2 and was calculated at the start of the study.

No insulin was administered during study periods. Short-acting insulin was withdrawn 12–14 h before the start of the study, intermediate-acting insulin at least 32 h before the start.

During both study periods the patients were offered identical diets with individually computed total caloric content, calculated from an ideal BMI of each patient (approximately 55% carbohydrate, 30% fat and 15% protein) and the level of exercise was the same in both periods (14). Before the start of the study all patients were informed about its design and possible risks. The protocol, which respected the principles of the Helsinki Declaration, was accepted by the Regional Ethical Committee.

Study design

The study was designed as a randomized, double-blind, placebo-controlled, cross-over study (14). The wash-out period between the two study periods in the hospital was at least 2 weeks. Each study period was 3 days, from Monday morning to Thursday morning. The tablets (125 mg acipimox or placebo) were taken every 2 h during the study period, from 0800 h on Monday to 0600 h on Thursday. The resulting plasma acipimox concentrations were above the level which effectively

suppresses lipolysis (10^{-5} mmol/l = $1.54 \mu\text{g/ml}$ (40)) in 243 out of 248 plasma acipimox concentrations from 1800 h on the first study day (14).

Blood samples were taken every 2 h throughout both study periods (first time Monday at 0800 h, last time Thursday at 0800 h) via a polyethylene catheter inserted into an antecubital vein.

Materials and analytical determinations

Plasma FFA, triglycerides, glucose, insulin, and acipimox levels were measured as previously described (14). Acipimox was supplied by Farmitalia Carlo Erba, Milan, Italy.

Plasma leptin levels were determined using the human leptin RIA kit with rabbit IgG raised against highly purified human leptin (Linco Research, Inc., St Charles, MO, USA). One missing measurement out of 592 was substituted by the mean of the two adjacent values.

Statistical analysis

Significance of effects of treatment was assessed with the Wilcoxon test for paired data. Due to the loss of 4 out of 16 body weight records, the Mann–Whitney test for unpaired data was used for body weights. *P* values less than 0.05 were considered significant.

Results

All patients lost body weight during the 3 days of hospitalization. There was no statistically significant difference between weight losses in the two periods (2.86 ± 0.40 kg ($n=7$) vs 1.97 ± 0.25 kg ($n=5$), NS (mean $\pm$ S.E. acipimox vs placebo)).

Acipimox lowered mean plasma glucose, insulin, triglycerides and FFA in all eight Type 2 diabetic patients during the 3 day treatment period. In Fig. 1 the area under the curve (AUC) is shown for glucose, insulin, triglycerides and FFA for each of the 3 days of either acipimox or placebo treatment.

The plasma leptin concentrations during acipimox and placebo treatment periods are shown with BMI for all eight individual subjects in Fig. 2. The leptin level at a given BMI is seen to be variable. Plasma leptin concentrations showed similar diurnal patterns in both acipimox- and placebo-treated patients with highest concentrations at 0400 h and lowest at 1400 h (Fig. 2). Simple inspection of the leptin data reveals that the relative amplitudes for leptin during acipimox treatment are not reduced compared with placebo treatment.

On day 1, plasma leptin concentrations were higher during acipimox treatment compared with placebo treatment in six out of the eight patients (Fig. 2). In the second half of day 2 and the whole of day 3, all eight patients had higher plasma leptin concentrations

Figure 1 The AUC (plasma concentration $\times$ 24 h) for glucose, insulin, triglycerides and FFA (mean $\pm$ S.E.) during 3 days of acipimox (grey bars) and placebo (white bars) treatment. * $P=0.05$, ** $P<0.05$, *** $P<0.001$.

during acipimox treatment compared with placebo (Fig. 2). The mean increase in plasma leptin levels during the 3 days of treatment was 2.38 ± 0.57 ng/ml, $P<0.005$.

In Fig. 3 the mean of the eight individual (24 h mean) relative treatment effects (placebo subtracted from acipimox treatment and divided by the individual mean) on plasma leptin, FFA, glucose and insulin are shown for each of the 3 study days. (This calculation was done to overcome the inter-individual differences in plasma leptin concentrations.) The effect of acipimox

on leptin concentration reached statistical significance on day 2 (0.17 ± 0.06 , $P<0.05$) and day 3 (0.25 ± 0.02 , $P<0.001$) and increased during the experimental period ($P<0.03$). The effect of acipimox on plasma glucose and insulin also reached statistical significance on day 2 (-0.23 ± 0.05 , $P<0.03$ and -0.31 ± 0.09 , $P<0.03$) and day 3 (-0.26 ± 0.05 , $P<0.03$ and -0.26 ± 0.10 , $P<0.05$). The pronounced fall in plasma FFA during acipimox treatment was statistically significant on days 1, 2 and 3 (-0.88 ± 0.06 , -0.79 ± 0.1 , -0.64 ± 0.07 , $P<0.001$). The drug effect decreased during the period (14).

Discussion

The main result of the study was the gradual increase in plasma leptin concentration during the 3 days of acipimox treatment.

Normally, the plasma leptin concentration correlates highly with BMI (41, 42). Moreover, women have higher plasma leptin concentrations than men, a relationship acipimox treatment did not change. All eight patients lost weight during the two study periods, and the weight loss was not significantly higher during acipimox treatment. During placebo treatment the trend in plasma leptin level was negative in accord with the loss of weight. During the 3 days of acipimox treatment the trend in plasma leptin was positive despite the weight loss. The presently observed diurnal variations are in accord with those previously found in rats and humans (31, 36). It has been reported that relative amplitudes for leptin concentrations in human subjects are decreased during reduced caloric intake (43, 44). During acipimox treatment the slightly higher weight loss did not affect the relative amplitudes for leptin concentrations.

The slow rise in leptin concentrations during acipimox treatment would be compatible with the notion that the rise was secondary to early changes in substrates or hormones induced by acipimox administration. However, the decreased plasma FFA, glucose and insulin would be expected to decrease leptin production.

The effect of FFA on leptin levels has been studied in rat tissue and human subjects. In rats, lipid infusion at the end of a 3 h euglycaemic, hyperinsulinaemic clamp was followed by an increase in leptin concentrations in plasma, adipose tissue and skeletal muscle (34). In accord with these results, 5 h of lipid infusion resulted in increased leptin mRNA concentrations in subcutaneous fat of healthy volunteers (35). In two other human studies no change in leptin levels was found after a short time (2 or 3 h) of lipid infusion (45, 46) or after an additional 3 h of lipid infusion in combination with a hyperglycaemic, hyperinsulinaemic clamp (45). Hennes *et al.* (45) found that acipimox treatment (1750 mg in 8 h) lowered FFA and increased plasma leptin concentrations after a hyperglycaemic,

Figure 2 Plasma leptin concentrations during 3 days of acipimox treatment in four male (A) and four female (B) Type 2 diabetic patients. Leptin concentrations were measured in blood samples taken every 2 h throughout the 3 days of treatment. Black lines indicate leptin concentrations during acipimox and grey lines indicate leptin concentrations during placebo treatment periods.

hyperinsulinaemic clamp. Those authors did not expand on the possibility that the increase in plasma leptin could be a primary effect of acipimox although FFA, insulin and glucose were kept constant.

It has been shown in human adipose tissue that acipimox mediates its antilipolytic action via inhibition of lipolysis (40). A mechanism shown in cultured adipocytes has been suggested: acipimox inhibits lipolysis through suppression of intracellular cAMP levels followed by decreased cAMP-dependent protein kinase activity (47). Acipimox may initiate its inhibition of lipolysis by binding to a G_i -protein-linked receptor in the plasma membrane (47). It is well described that catecholamines increase cAMP concentration and thereby lipolysis in fat tissue (48). Moreover, it has been reported that catecholamines inhibit leptin secretion in

human subjects (49). Thus, it could be suggested that changes in cAMP concentration in adipocytes regulate leptin secretion and that the suppression of cAMP caused by acipimox treatment results in increased leptin production. This hypothesis is further supported by the fact that insulin inhibits lipolysis in adipocytes, decreases cAMP concentration (50, 51) and also increases leptin production (see below). Thus, a common messenger for the inhibition of lipolysis by acipimox and insulin may be cAMP.

It has been described in several *in vitro* studies that insulin stimulates leptin secretion (36–38). In human subjects an increase in leptin levels was found after supra-physiological insulin infusions for 4 h (39) and after physiological insulin infusions for 6 h (32). Others have, however, not been able to demonstrate an effect

Figure 3 The mean of the eight individual (24 h mean $\pm$ S.E.) relative treatment effects (placebo subtracted from acipimox treatment and divided by the individual mean) on plasma FFA, glucose, insulin and leptin for each of the 3 study days. * $P < 0.05$, ** $P < 0.03$, *** $P < 0.001$.

of insulin on plasma leptin concentrations in human subjects after 5 or 6 h of either supra-physiological or physiological infusions (38, 52). Following glucose or glucosamine infusion in combination with infused somatostatin, Wang *et al.* (34) found an increase in ob mRNA and leptin in adipose tissue in rats, and they also found that the very low levels of ob mRNA in skeletal muscle were increased. Thus, increased plasma glucose may also stimulate leptin secretion. In the present study, however, the plasma leptin concentration increased despite decreased plasma insulin as well as plasma glucose.

The gradual and parallel fall in plasma glucose and plasma insulin during acipimox treatment indicates improved insulin sensitivity. The fall parallels the gradual rise in plasma leptin over time. It is possible that the increased plasma leptin level contributes to the increased glucose uptake since it has been shown that leptin stimulates glucose uptake in muscle. In myotubes, leptin stimulated glucose uptake and glycogen synthesis (53). In wild-type mice i.v. infusion of leptin for 3–5 h resulted in increased glucose turnover, glycolysis, glucose uptake and glycogen synthesis in

muscle (54). Thus, it cannot be excluded that the present increase in leptin concentration contributes to an increased glucose uptake in skeletal muscle. Several studies have shown that leptin inhibits insulin secretion (55–58). In isolated islets from ob/ob mice it was found that leptin suppressed insulin secretion by the activation of ATP-sensitive potassium channels (57) and in human pancreatic islets insulin secretion and gene expression were markedly decreased after leptin administration (58). In the present study, the decreased plasma insulin may be sufficiently explained by the decrease in plasma glucose; however, we cannot exclude a contributory suppression by leptin.

We conclude that 3 days of intensive treatment with the antilipolytic drug acipimox results in an increasing rise in plasma leptin concentration together with improved insulin sensitivity (lowering of plasma FFA, triglycerides, glucose and insulin) in Type 2 diabetic patients. Thus, results of previous studies indicating that leptin induces insulin resistance and may be involved in the development of the diabetic syndrome (27–29, 55) cannot be supported by the present results. It cannot be excluded that acipimox increases leptin production via fat accumulation (59). It is also possible, however, that the increased plasma leptin concentration is a primary acipimox effect which prevails over a reduced FFA stimulus and may contribute to increased glucose uptake in skeletal muscle. In the latter case the cAMP concentration in adipocytes may mediate the effect of acipimox on leptin secretion (48, 49).

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