

Original Article

The effect of pH-neutral peritoneal dialysis fluids on adipokine secretion from cultured adipocytes

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Abstract

Background. Adipokines are a group of fat-secreted hormones and cytokines, including leptin and adiponectin, with important functions in humans. Peritoneal dialysis (PD) is associated with markedly raised plasma adipokines, suggesting increased production in this setting. We have shown that low pH down-regulates leptin production. The current study was designed to test if novel pH-neutral PD fluids may regulate leptin and adiponectin secretion *in vitro*.

Methods. We exposed 3T3-L1 adipocytes to a 50:50 mixture of dialysate and M199 containing 10% serum for up to 48 h. Dialysates were commercial PD fluids, i.e. conventional acidic, lactate-buffered solutions (PD-acid) and pH-neutral lactate-buffered (PD-Bal) or bicarbonate-buffered solutions (PD-Bic). Leptin and adiponectin concentrations in culture-cell media were measured by ELISA.

Results. Compared with PD-acid, PD-Bal and PD-Bic produced a 25 and 43% increase, respectively, in leptin secretion at 48 h ($P < 0.05$). In contrast, adiponectin secretion was not affected. High glucose PD fluids (4.25%) specifically inhibited leptin secretion vs 1.5% glucose, buffer-matched solutions ($P < 0.05$). However, differences in leptin secretion due to pH and type of buffer remained significant. In further experiments, the pH of test media were extensively varied without the presence of dialysates. Leptin secretion was shown to increase in a parallel to pH, whereas large changes in pH did not affect adiponectin secretion.

Conclusion. The pH-neutral PD solutions specifically induce leptin, but not adiponectin secretion from 3T3-L1 adipocytes. PD-Bic produced a greater leptin stimulation than PD-Bal, but this difference was attributable to pH *per se*, rather than the type of buffer.

Keywords: adipocytes; adipokines; adiponectin; leptin; PD fluids; pH

Introduction

The adipose tissue is now recognized as an active endocrine organ, secreting a number of hormones and cytokines, called adipokines. Leptin and adiponectin are adipokines with well-defined actions in humans. Leptin is a 167-amino-acid circulating protein encoded by the *ob* gene, which signals the body's nutritional status to the brain to regulate energy balance [1]. It also has distinct cytokine properties, such as the stimulation of inflammation and modulation of the immune system [2]. Adiponectin is a 244-amino-acid protein produced by the *apM1* gene, which recently has attracted major attention because of its anti-inflammatory, insulin-sensitizing and anti-atherogenic properties [3].

Peritoneal dialysis (PD) is characterized by fat accumulation, markedly raised plasma leptin [4] and moderate hyperadiponectinaemia [5] and, thus, represents a distinct condition for the study of adipokine metabolism. The clinical significance of an excess of adipokines in the plasma of PD patients is yet to be determined. However, relationships between plasma adipokines and clinical outcome have been recently demonstrated in other settings. For instance, hyperleptinaemia has been independently associated with the occurrence of a first myocardial infarction in the general population [6]. Moreover, patients on haemodialysis (HD) with plasma adiponectin lower than 11.6 µg/l were reported to have a worse 3-year survival, compared with patients with higher levels [7]. Significant amounts of adipokines are measured in the peritoneal cavity of PD patients, suggesting active local secretion (personal unpublished observations). During the dwell, solutes in PD fluids are actually transferred by passive diffusion through the peritoneal membrane [8], and hence come into contact with intra-abdominal adipose tissue,

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a source of adipokine synthesis. It is conceivable that adipokines, locally secreted in response to PD fluids, may contribute to pathophysiological changes of the peritoneal membrane, through defined biological actions. In the last few years, conventional acidic lactate-based PD fluids have been progressively replaced by more biocompatible pH-neutral PD solutions. We have previously demonstrated that a low pH reduced leptin production *in vitro* [9] and systemically in uraemic rats [10]. Thus, we propose that novel pH-neutral PD fluids may have an impact on adipokine production from adipose tissue. This hypothesis was tested *in vitro* by employing the 3T3-L1 adipocyte cultured-cell model, that has been abundantly characterized for studies regarding leptin secretion in this laboratory [9–11].

Materials and methods

Dulbecco's modified Eagle's medium (DMEM) (Gibco, BRL, Life Technologies, 31885-023, Paisley, UK) was used as growth medium for cell cultures. Medium M199 Earl's salt (Gibco, BRL, Life Technologies, 31150-022, Paisley, UK) and minimal essential medium (MEM) (Gibco, BRL, Life Technologies, 32571-028, Paisley, UK) were employed as test media. All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Seromed S0115, Fakola AG, Basle, Switzerland), and 1% penicillin/streptomycin (10 000 U/10 000 µg/ml); (Invitrogen, Basle, Switzerland). Insulin, dexamethasone and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma-Aldrich; and sodium pyruvate from Invitrogen (Basle, Switzerland). Cell-culture plastics were supplied by Falcon (Geneva, Switzerland).

Choice of *in vitro* model

In the design of this study, four *in vitro* models of adipose tissue were tested. Human omental adipocytes were isolated by collagenase digestion from omental biopsies from uraemic PD patients undergoing catheter insertion. The viable non-adherent adipocyte suspensions obtained were unsuitable however, as viability declined after only 12 h in suspension. In contrast, preadipocytes isolated from the aforementioned biopsies were adherent to plastic flasks, proliferated and differentiated in culture. However, the small number of cells available from each tissue donor precluded their use in routine experiments. Omental adipose tissue explants (2–5 mm in diameter) obtained from further donors were also cultured for up to 48 h and showed significant, but widely varying, leptin secretion, owing to wide variations in the ratio of adipose to connective tissue in the samples. The present study was therefore performed in the 3T3-L1 preadipocyte culture model. This cell line has several advantages over the aforementioned culture-cell models: It represents a homogeneous cell population, allowing precise comparisons of defined treatments, without the confounding factor of variation between different donors. It has been successfully used to study the effects of pH on leptin [9,10]. Finally, when subjected to changes in glucose availability, leptin secretion responds in a comparable manner in 3T3-L1

adipocytes [11], epididymal rat [12] and human subcutaneous adipocytes in culture [13], suggesting that differences in the leptin response to nutrients between adipocyte cell types or lines are unlikely.

The 3T3-L1 fibroblasts were grown to confluence in DMEM, and differentiation to adipocytes was stimulated by incubation with isobutylmethylxanthine, dexamethasone and insulin, as described previously [9–11]. At this stage, phase contrast microscopy showed that all the cells exhibited typical adipocyte morphology without any apparent fibroblast contamination. Terminal differentiation of the 3T3-L1 preadipocytes was monitored by washing the cells in phosphate-buffered saline (PBS), followed by fixation in 3.7% formaldehyde/PBS for 10 min, staining with Oil-Red-O dye for fat droplets for 1 h, and observation under a phase contrast microscope. Bright staining of the cytoplasm indicated that accumulation of great amounts of lipids had occurred and thus, adipocyte differentiation was achieved. Experiments were only performed if this feature was present in more than 98% of the cells.

Experimental design

After complete differentiation, 3T3-L1 adipocytes were rinsed and exposed to a 50:50 mixture of the PD-dialysis solution and M199 with 10% FBS, 1000 IU/ml penicillin, 1000 µg/ml streptomycin and 1 mmol/l sodium pyruvate. The dilution of PD fluid in this way, described previously [11], was used to mimic the equilibration of dialysis solutions occurring early after their infusion into the peritoneal cavity, i.e. a fall in glucose and lactate concentrations and osmolality, and an increase in pH. Culture plates were incubated at 37°C in 5% CO₂. Aliquots of cell-culture media were collected at 48 h. This timing was chosen according to previous investigations showing that pH induces significant effects on leptin secretion *in vitro* at this time point [9,10]. The samples were frozen at –20°C for subsequent leptin and adiponectin assays. Cell monolayers were lysed by scraping the cells from the culture plate into 0.5 M sodium hydroxide. Intracellular total protein was measured using a modified Lowry technique (BioRad DC protein assay, BioRad).

The PD fluids tested in this study were commercial dialysates provided by Fresenius Medical Care, Bad Homburg, Germany. They were allocated into two groups according to their glucose concentrations of 1.5 and 4.25%, respectively. In each group, a PD-acid was compared with a neutral-pH solution, PD-Bal or PD-Bic. A solution made of M199 containing 5% FBS with the same additions as aforementioned was used as the control (Ctrl). The precise compositions of PD fluids are given in Table 1. Confounding factors were avoided, as PD fluids to be compared had identical concentrations of sodium, calcium and magnesium and similar osmolalities. Differences in chloride concentrations were minimal. pH-neutral fluids were manufactured with separate alkaline and acidic fluid compartments. This allowed the sterilization of glucose at very low pH, with greatly reduced glucose degradation product (GDP) formation, to finally produce neutral-pH dialysis solutions.

Each set of experiments was performed with the entire panel of PD fluids plus the Ctrl medium in at least one well in each plate. Each plate was reproduced at least in triplicate.

Table 1. Chemical composition of PD test solutions

PD solutions (study code number)	[Glucose] 1.5%			[Glucose] 4.25%		
	CAPD/DPCA 2 <i>Stay-Safe</i> [®] (PD-acid 1.5)	CAPD/DPCA 2 <i>Stay-Safe-Balance</i> [®] (PD-Bal 1.5)	CAPD/DPCA <i>Bicavera</i> [®] (PD-Bic 1.5)	CAPD/DPCA 3 <i>Stay-Safe</i> [®] (PD-acid 4.25)	CAPD/DPCA 3 <i>Stay-Safe-Balance</i> [®] (PD-Bal 4.25)	CAPD/DPCA <i>Bicavera</i> [®] (PD-Bic 4.25)
Theoretical pH	≈5.5	≈7.0	≈7.4	≈5.5	≈7.0	≈7.4
Measured pH	5.65	7.40	7.43	5.44	7.21	7.30
Osmolality (mOsm/l)	≈358	≈358	≈358	≈511	≈511	≈510
[Na ⁺] (mM)	134	134	134	134	134	134
[Ca ⁺⁺] (mM)	1.75	1.75	1.75	1.75	1.75	1.75
[Mg ⁺⁺] (mM)	0.5	0.5	0.5	0.5	0.5	0.5
[Cl ⁻] (mM)	103.5	101.5	104.5	103.5	101.5	104.5
[Lactate ⁻] (mM)	40	35	–	40	35	–
[Bicarbonate ⁻] (mM)	–	–	34	–	–	34

Leptin and adiponectin protein quantitation

Leptin concentrations in cell-culture media were determined using a commercial sensitive and specific sandwich ELISA for mouse leptin (R&D Systems, Quantikine Elisa Kit, Minneapolis, MN, USA), as described previously [9–11]. The assay is able to detect minimal leptin concentrations of 22 pg/ml.

Adiponectin concentrations in cell-culture media were determined using a sensitive and specific sandwich ELISA for mouse adiponectin (R&D Systems, Quantikine Elisa Kit, Minneapolis, MN, USA), following a similar layout. The minimal adiponectin concentration detectable by the assay is 15.6 pg/ml. The ELISA is linear within the range 0.25–8.0 ng/ml.

pH measurements

The pH from fresh dialysates and final test media were measured in triplicate using a pHmeter (Model 632, Metrohm, Herisau, Switzerland). Fresh Dialysates were extracted from the bags with a syringe and then transferred in Falcon tubes which were immediately closed with plastic hubs. After a short equilibration to room temperature of 25°C, the tubes were opened and pH was stabilized 10 s before measurements. Mixed solutions including fresh dialysates extracted as aforementioned and freshly opened M199 were prepared in the incubator and collected in Falcon tubes, which were subsequently closed. The pH measurements at time 0 were performed after transfer on the bench, as described earlier in this article, i.e. avoiding significant contact with the air atmosphere to prevent loss of CO₂ and subsequent pH rise. The pH levels of these solutions were also measured after 24 and 48 h of incubation, with and without adipocytes, using a similar method.

Modulation of adipokine secretion by changes of pH

Further experiments were performed to test whether large changes of pH may modulate adipokine secretion, independently of the presence of dialysates or the type of buffer. For this purpose, MEM with 5% heat inactivated FBS with additions as aforementioned, was used as a test medium. The pH was varied over a wide range (7.1–8.5) by addition of HCl or NaHCO₃, as described previously [9]. NaCl was

added to the acidic cultures to maintain a constant Na concentration.

Statistical analyses

Results were expressed as values normalized to the response obtained with the M199 Ctrl medium. This method allowed comparisons between experiments done at different times regardless of differences in the amount of adipocytes present in different sets of experiments. Mean percentages of change ± SEM were reported, unless otherwise specified. Statistics were established with GraphPad Prism, version 4.00, for Windows. Comparisons between group analysis were performed using a one-way analysis of variance (ANOVA) followed by a Dunnett's post-test to compare all the columns with the Ctrl or Bonferroni's test to compare the stimulating/inhibiting effect of selected pairs of media. A probability value of $P < 0.05$ was considered to indicate statistical significance.

Results

Assessment of cell model after challenge with PD fluids

Previous experiments from our group already showed that conventional lactate-based PD fluids, such as PD4, and laboratory-manufactured dialysates did not affect cell mass, number and adipocyte functions [11]. In addition, the percentage of LDH release in the cell supernatants was 0.2 to 0.3%, indicating that these dialysates did not induce significant toxicity on 3T3-L1 adipocytes [11]. In the current study, neither conventional solutions PD-acid, nor pH-neutral PD-Bal and PD-Bic solutions affected intracellular protein content, when compared with the Ctrl medium. In addition, cell counts after vital stain coloration (trypan blue exclusion) did not differ. This corroborates the consistency and reliability of this cell model regarding the response to PD solutions.

Effect of pH-neutral PD fluids on leptin secretion

Compared with PD-acid, the pH-neutral dialysates PD-Bal and PD-Bic produced an increase in leptin

secretion of $25 \pm 2\%$ and $43 \pm 4\%$ at 48 h ($P < 0.05$ and < 0.01 , respectively) (Figure 1A), PD-Bic being slightly, but significantly more effective than PD-Bal in stimulating leptin production ($P < 0.05$).

Effect of high glucose-based PD fluids on leptin secretion

High glucose-based PD fluids (4.25%) significantly inhibited leptin release vs PD fluids with a glucose concentration of 1.5% ($P < 0.05$). However, the leptin-stimulating effect by pH-neutral solutions, and the differences between PD-Bal and PD-Bic remained significant (Figure 1B).

No effect of pH-neutral PD fluids on adiponectin secretion

In contrast to leptin, adiponectin secretion was not affected by pH-neutral PD fluids (Figure 2), nor by PD solutions containing high glucose concentrations.

pH measurements

According to the manufacturing company, the pH of PD-acid, PD-Bal and PD-Bic were approximately 5.5, 7.0 and 7.4, respectively. These values were confirmed by pH measurements from fresh dialysates (Table 1). After mixing with M199, the pH of all test media were higher, as shown in Table 2. This was due to the important buffering capacity of M199. However, PD-Bal and PD-Bic fluids still showed significantly higher pH ($P < 0.01$) than PD-acid. As expected,

the highest pH values were measured in PD-Bic ($P < 0.05$ vs PD-Bal).

After 24 and 48 h of incubation with cultured adipocytes, fluctuations in pH were noticed. In particular, PD-Bal became more acidic ($P < 0.01$) and the differences in pH between PD-acid and PD-Bal tended to decrease. In addition, pH of PD-Bic solutions tended to increase especially in high glucose solution ($P < 0.01$). It is noteworthy that the presence vs absence of adipocytes during incubations did not affect pH values (data not shown).

Relationship between pH and adipokine secretion

The relationship between the pH of PD fluids and leptin secretion was studied by plotting leptin secretion data at 48 h against mean pH measurements during 48 h of incubation with cells (mean out of three pH values). Leptin secretion increased in parallel to pH increments (Figure 3).

In further experiments, pH was varied over a larger range (7.1–8.5) in test medium MEM containing 5% FBS. The pH of MEM was either decreased by adding HCl or increased by supplementation of NaHCO_3 . This method allowed to study the effect of pH *per se*, thus avoiding the confounding factor due to the type of buffer. Again, leptin secretion at 48 h increased in parallel to increments of pH, indicating that pH, rather than the type of buffer, was responsible for the leptin-stimulating effect of pH-neutral PD fluids. Adiponectin secretion was not affected by large changes in pH (Figure 4), which was consistent with experiments using pH-neutral dialysis fluids.

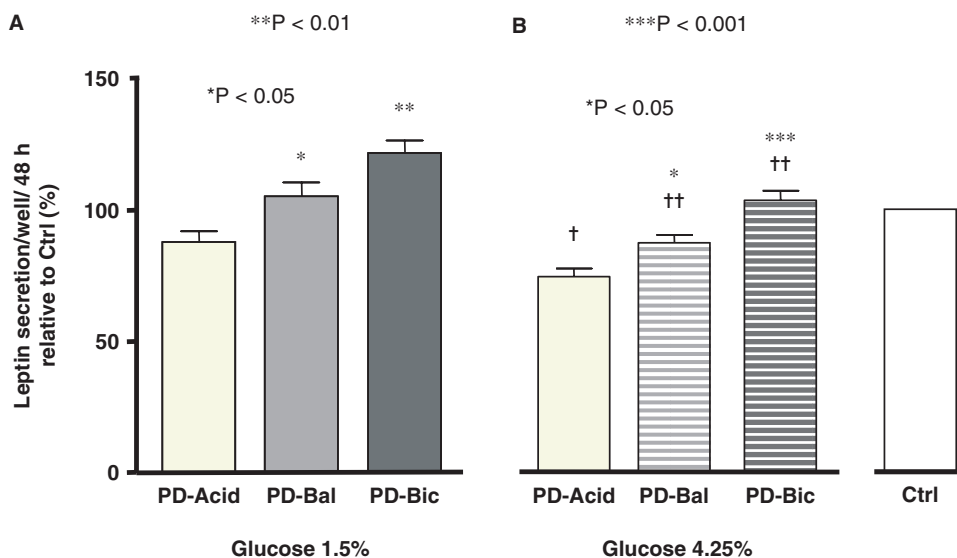


Fig. 1. Effect of pH-neutral PD fluids on leptin secretion. The 3T3-L1 adipocytes in six-well plates were incubated for 48 h in PD-acid, PD-Bal or PD-Bic at glucose concentrations of 1.5 g/dl (Panel A) or 4.25 g/dl (Panel B). Solutions were diluted 50:50 with M199. Results are expressed as mean \pm SEM percentages of increase relative to M199 (Ctrl). Results shown are those obtained from eight experiments (pool of 40–48 wells per condition). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs PD-acid. † $P < 0.05$; †† $P < 0.01$ vs respective buffer-matched PD fluids with glucose 1.5%.

Discussion

This study shows that pH-neutral PD fluids specifically stimulate leptin, but not adiponectin secretion from 3T3-L1-cultured adipocytes. A greater leptin-stimulating effect was observed with bicarbonate-based vs lactate-based pH-neutral PD fluids, but this difference appeared to be induced by pH, rather than the type of buffer.

When PD solutions are used as test media, numerous chemical components beyond pH or buffers, may produce significant biological effects. However in this study, confounding factors have been carefully avoided. The solutions tested were absolutely comparable regarding electrolyte and glucose concentrations. GDP content, which is strongly reduced in PD-Bal and PD Bic, in comparison with conventional PD fluids such as PD-acid, might theoretically have affected adipokine production. However, we have already demonstrated that adipocytes exposed to commercial dialysates containing high GDP contents produce similar leptin secretion rates than sterile-filtered,

i.e. GDP-free, comparable solutions [11]. Thus, differences in GDP content could not be accounted for by changes in leptin secretion observed in the current study. As a result, the leptin-stimulating effect by pH-neutral PD fluids could readily be ascribed to neutralization of PD fluids.

We have previously shown that a low pH of 7.1 down-regulates leptin secretion through inhibition of glucose transport [10]. However, the current study has examined this effect in the specific context of PD fluids, i.e. in highly unphysiological conditions. As shown in Table 2, our experimental model enabled the generation of pH conditions *in vitro* remarkably similar to those observed in the peritoneal cavity of PD patients. In spite of the buffering effect of M199, pH differences between test solutions persisted. Whether the higher pH of pH-neutral PD fluids or the specific type of buffer were responsible for the leptin-stimulating effect was questioned. For this purpose, we tested if leptin secretion was pH-dependent over a wide range, independently of the presence of dialysis fluids. As shown with pH-neutral

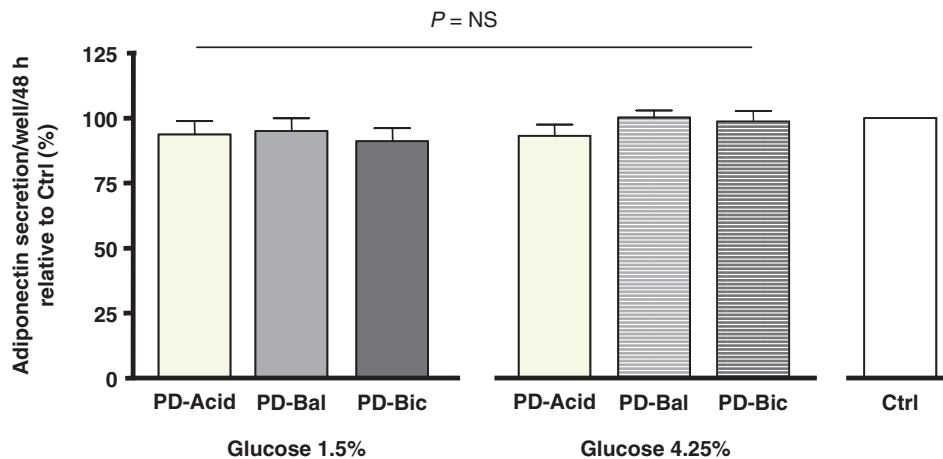


Fig. 2. Effect of PD neutral-pH PD fluids on adiponectin secretion. The 3T3-L1 adipocytes were incubated for 48 h in PD-acid, PD-Bal or PD-Bic at glucose concentration of 1.5 or 4.25 g/dl. Solutions were diluted 50:50 with M199. Results are expressed as mean \pm SEM percentages of secretion rates, relative to M199 (Ctrl). Results shown are those obtained from eight experiments (pool of 40–48 wells per condition).

Table 2. Measurements of pH of PD fluids, after mixing with M199

PD-fluids	t = 0	t = 24 h	t = 48 h
PD-acid 1.5 (mean \pm SEM)	7.13 \pm 0.02	7.04 \pm 0.02	7.07 \pm 0.01
PD-Bal 1.5	7.19 \pm 0.02*	7.08 \pm 0.02	7.11 \pm 0.02
PD-Bic 1.5	7.24 \pm 0.02**	7.44 \pm 0.03**	7.22 \pm 0.03**
PD-acid 4.25	7.12 \pm 0.02	7.03 \pm 0.03	7.04 \pm 0.01
PD-Bal 4.25	7.19 \pm 0.01*	7.10 \pm 0.02*	7.09 \pm 0.01*
PD-Bic 4.25	7.22 \pm 0.03**	7.41 \pm 0.05**	7.49 \pm 0.03**
M199 Ctrl	7.19 \pm 0.02	7.33 \pm 0.03	7.71 \pm 0.03

The pH of M199 alone and of the final test media, i.e. mix between fresh dialysates and M199, were measured out of the incubator, at room temperature of 25°C, in a way to avoid contact with the atmosphere, as described in the method's section. Time 0 (t=0) means that pH measurements were made immediately after the solutions, prepared in the incubator, and transferred on the bench. T = 24 h and t = 48 h indicate that pH were assessed as aforementioned after solutions have been incubated during 24 and 48 h, respectively.

Results are mean \pm SEM of triplicates.

* $P < 0.05$; ** $P < 0.01$ vs PD-acid for the same glucose concentration.

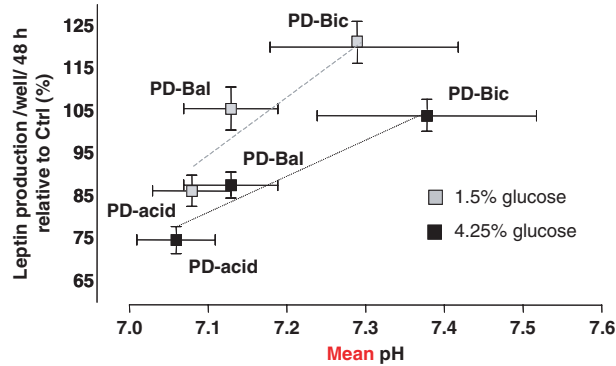


Fig. 3. Relationship between the pH of PD fluids and leptin secretion. Leptin secretion rates relative to control, at 48 h, were plotted against mean pH value of PD fluids measured during a 48 h course of incubation with the cells. The pH measurements were determined in triplicate, immediately after PD fluids were mixed 50:50 with M199, at 24 and 48 h. The mean \pm SEM of these pH values is shown.

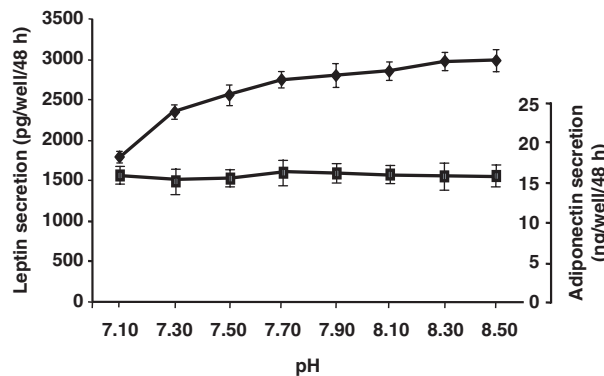


Fig. 4. The effect of pH on adipokine secretion. MEM containing 5% FBS, with a glucose concentration of 5.5 mM, was used as a test medium. The pH was varied by addition of HCl or NaHCO₃. The pH measurements did not significantly vary over the incubation period. Leptin [◆] and adiponectin [■] concentrations were assayed in culture-cell media. Results shown are those from pH at the start of the incubation period (x axis) and leptin/adiponectin secretion (y axis) obtained after 48 h of incubation (mean \pm SEM of four experiments, pool of 31–35 culture wells for each set of conditions).

PD fluids, leptin secretion increased gradually in parallel to pH increments. These data strongly suggest that the leptin-stimulating effect of pH-neutral PD fluids was attributable to pH *per se*, rather than the type of buffer.

The precise mechanism responsible for this effect was not investigated in the present study. However, high pH has been shown to increase glucose transport in rat adipocytes [14]. Furthermore, we have demonstrated that reduced glucose influx into adipocytes was the precise mechanism by which a low pH inhibited leptin secretion, at post-transcriptional level [10]. So, we suggest that pH-neutral solutions may increase glucose transport and, hence, stimulate leptin secretion.

The observation that adiponectin secretion was not regulated by pH and/or pH-neutral PD fluids represents an additional novel finding of this study. Molecular mechanisms regulating adiponectin

secretion have been recently reviewed [15]. Adiponectin is negatively regulated by accumulation of fat mass, glucocorticoids, β -adrenergic agonists and tumour necrosis factor (TNF- α). In contrast, up-regulation of adiponectin has been reported with substantial weight loss and the use of thiazolidinediones. The effect of insulin *per se* on adiponectin protein secretion and gene expression is controversial, since both positive and negative regulations have been observed [15]. It was recently evidenced that the activation of the hexosamine signalling pathway resulted in decreased serum adiponectin in mice [16]. So, we expected that high pH would affect adiponectin secretion, through increased glucose transport into the cell, and hence glucose flux into the hexosamine pathway. As this was not the case, further studies are needed to investigate this issue.

Dialysates with the highest glucose concentration (4.25%) inhibited leptin secretion compared with solutions containing a lower glucose concentration (1.50%). This finding was apparently at odds with our former report showing that increments of glucose concentration from 2.5 to 40 mM increased leptin secretion in a dose-dependent fashion [11]. It has to be emphasized that extremely high glucose concentrations, as those applied to these cells in the current study (4.25% solutions produced an extracellular glucose concentration of 124 mM after dilution with M199) are known to deplete intracellular adenosine triphosphate, which in turn suppress leptin secretion [17]. So, it is suggested that up-regulation of leptin by extracellular glucose is limited to a physiological range of glucose concentrations, beyond which leptin secretion is suppressed. Nevertheless, in spite of this inhibition, the leptin-stimulating effect by pH-neutral PD fluids was still significant. Finally, PD-acid did not produce a higher leptin secretion than M199 (Figure 1A), despite containing a higher glucose concentration (45 vs 5 mM). This apparent lack of leptin-stimulating effect by high glucose may be explained by a counteracting effect of the lower pH of PD-acid (7.1 vs 7.4). PD-Bic, a solution with a comparable pH but a higher glucose concentration than M199, actually induced a 25% increase in leptin secretion, which is consistent with this hypothesis. Thus, it is proposed that both glucose and pH from PD fluids may act in concert to regulate leptin production *in vitro*. Whether one of these factors may predominate over the other to determine leptin levels in PD patients needs to be clarified in clinical studies.

In contrast to leptin, high glucose-based dialysate concentrations did not affect adiponectin secretion. We previously observed that, in 3T3-L1 adipocytes, glucose-based dialysis solutions (glucose 1.36%) reduced adiponectin secretion vs glucose-free PD solutions (amino-acid and icodextrin solutions) (unpublished data). Thus, the regulation of adiponectin secretion by extracellular glucose appears to occur within limits in the range of glucose concentrations.

Leptin has pleiotropic peripheral effects, well beyond the regulation of energy balance. In particular, leptin stimulates atherogenesis, angiogenesis and inflammation *in vivo* [18]. Leptin also modulates T-cell-mediated immune responses and stimulates phagocyte function [19]. Thus, the issue of marked hyperleptinaemia observed in PD patients is relevant. We have recently reported that glucose concentration in PD fluids stimulates leptin production from cultured adipocytes *in vitro* [11]. Leptin concentrations in PD effluents are higher than would be expected by its molecular weight [20]. In some cases, intraperitoneal levels may exceed circulating levels (personal unpublished observations). Therefore, locally produced leptin may possibly be transferred from the peritoneal cavity to systemic circulation, thus explaining the dramatic rise in plasma leptin observed in patients after the start of PD treatment. The current *in vitro* data suggest that further induction of plasma leptin by pH-neutral PD fluids is possible. This hypothesis is currently investigated in our centre.

The clinical relevance of leptin and other adipokines in the peritoneal cavity from PD patients is still uncertain. There is evidence that pH-neutral solutions have improved biocompatibility and preserve the peritoneal membrane. So, an increased local production of leptin by adipocytes exposed to pH-neutral solutions may reflect a state of better biocompatibility, which may primarily serve to improve local host defence. Similarly, pH-neutral PD fluids were also found to increase, and not reduce, TNF- α production by *ex vivo* peritoneal macrophages [21], which supports this view. However, it is unclear whether this beneficial effect would predominate over potential pro-inflammatory and angiogenic actions that leptin may have on the peritoneal membrane. Further research is warranted to provide additional knowledge in this area.

Limitations of the study

It could be argued that the 3T3-L1 preadipocyte culture model has some limitations in studying the response of omental adipocytes to PD fluids. This is a mouse and not a human model of adipose cells. Furthermore, 3T3-L1 adipocytes appear more similar to subcutaneous than visceral adipocytes. However, we and others have shown that leptin responses to various nutrient stimuli are highly conserved across several adipocytes cell types or lines [9–14]. It is therefore unlikely that, adipocytes from other sources, e.g. visceral adipocytes from PD patients grown in isolation *in vitro*, would have provided a more accurate model than 3T3-L1 adipocytes for this investigation. The observation that PD fluids did not induce toxicity or inhibitory effect in this cell model is not in agreement with studies on other cell types including human peritoneal mesothelial cells [22]. Nevertheless it is unclear whether this apparent resistance of 3T3-L1 adipocytes

to PD fluids is a true limitation of this model or is representative of adipocytes in general. Unfortunately, technical setbacks experienced with cultures of human primary adipocytes or fat explants isolated from uraemic patients did not allow to clarify this issue.

In summary, we have demonstrated that pH-neutral PD fluids regulate the adipokine production balance *in vitro*, in cultured 3T3-L1 adipocytes, by specifically inducing leptin, without affecting adiponectin production. This effect appears to be pH-dependent, which further consolidate the important role of pH in regulating leptin production.

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