Apelin stimulates glucose uptake but not lipolysis in human adipose tissue *ex vivo*

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Abstract

Apelin is a peptide present in different cell types and secreted by adipocytes in humans and rodents. Apelin exerts its effects through a G-protein-coupled receptor called APJ. During the past years, a role of apelin/APJ in energy metabolism has emerged. Apelin was shown to stimulate glucose uptake in skeletal muscle through an AMP-activated protein kinase (AMPK)-dependent pathway in mice. So far, no metabolic effects of apelin have been reported on human adipose tissue (AT). Thus, the effect of apelin on AMPK in AT was measured as well as AMPK-mediated effects such as inhibition of lipolysis and stimulation of glucose uptake. AMPK and acetyl-CoA carboxylase phosphorylation were measured by western blot to reflect the AMPK activity. Lipolysis and glucose uptake were measured, *ex vivo*, in response to apelin on isolated adipocytes and explants from AT of the subcutaneous region of healthy subjects (body mass index: 25·6 $\pm$0·8 kg/m^2, n=30 in total). *APJ* mRNA and protein are present in human AT and isolated adipocytes. Apelin stimulated AMPK phosphorylation at Thr-172 in a dose-dependent manner in human AT, which was associated with increased glucose uptake since C compound (20 μM), an AMPK inhibitor, completely prevented apelin-induced glucose uptake. However, in isolated adipocytes or AT explants, apelin had no significant effect on basal and isoprenaline-stimulated lipolysis. Thus, these results reveal, for the first time, that apelin is able to act on human AT in order to stimulate AMPK and glucose uptake.

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Introduction

Apelin is a peptide, identified as the endogenous ligand of APJ, a ubiquitously expressed G-protein-coupled receptor (Tatemoto *et al.* 1998). Apelin is synthetized as a 77-amino-acid prepropeptide, which is cleaved in different fragments including apelin-36, apelin-17, apelin-13, and the posttranslationally [Pyr1]apelin-13 with a conversion of the N-terminal glutamate to pyroglutamate preventing enzymatic breakdown and thus preserving biological activity (Tatemoto *et al.* 1998). Prior to being revealed as an adipocyte-secreted factor (adipokine), apelin was known not only to exert several central and peripheral effects on different tissues such as the regulation of the cardiovascular, immune, and gastrointestinal functions but also on fluid homeostasis, angiogenesis, proliferation of different cell types, and embryonic development (Kleinz & Davenport 2005). Recently, we have demonstrated that i.v. injection of apelin, at physiological dose, was able to decrease glycemia in mice (Dray *et al.* 2008). Moreover, during a hyperinsulinemic-euglycemic clamp, an increased glucose uptake was observed in adipose tissue (AT) and skeletal muscle (Dray *et al.* 2008). Apelin-stimulated glucose transport in soleus muscle was dependent on AMP-activated protein kinase (AMPK) activation (Dray *et al.* 2008). A similar stimulation of AMPK by apelin has also been described in cultured C2C12 myotubes (Yue *et al.* 2009). Taken together, these independent observations support a physiological role of apelin in glucose metabolism. Recent data have also highlighted an important role for apelin in lipid metabolism. Higuchi *et al.* (2007) showed that prolonged treatment of apelin (daily i.p. injection of apelin for 2 weeks) decreased the triglycerides content of AT and the weight of different fat depots in chow-fed and obese mice. In addition, changes in uncoupling protein-1 (UCP-1) expression in brown AT as well as changes in UCP-3 expression in skeletal muscle were observed after apelin treatment but no metabolic effects were studied (Higuchi *et al.* 2007).

In humans, different studies have reported changes in plasma apelin concentration and variations of apelin expression in different tissues between physiological and pathological situations (Carpéné *et al.* 2007). Insulin has been shown to be one of the main regulating factors of apelin expression and secretion (Boucher *et al.* 2005). Circulating apelin levels as well as AT expression increase in obese and hyperinsulinemic subjects (Boucher *et al.*

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2005, Castan-Laurell *et al.* 2008). Plasma apelin levels are also raised in morbidly obese (Heinonen *et al.* 2005) and type 2 diabetic (Soriguer *et al.* 2009) subjects. Nonobese patients with impaired glucose tolerance or with type 2 diabetes also exhibited higher concentrations of apelin when compared with control subjects (Li *et al.* 2006). The high apelin concentrations can be modulated by weight loss. Indeed, it was shown that diet-induced weight loss reduces plasma apelin levels in women with moderate obesity (Castan-Laurell *et al.* 2008), but not significantly in patients with metabolic syndrome (Heinonen *et al.* 2009). Bariatric surgery leads to a significant decrease in plasma apelin in morbidly obese subjects with impaired fasting glucose or type 2 diabetes before surgery (Soriguer *et al.* 2009). Moreover, the combination of two antidiabetics such as metformin and rosiglitazone in patients with type 2 diabetes improves glycemic profile and allows an increase in plasma apelin concentrations (Kadoglou *et al.* 2010). Altogether these studies suggest that the increased levels of apelin might be, in early stage of metabolic disease, a compensatory mechanism delaying the onset of insulin resistance.

So far, to our knowledge, metabolic effects of apelin on human AT and the effect of apelin on AMPK activity in AT have not been published. Adipocytes are involved in lipid storage (lipogenesis) through lipid and glucose uptake but also in triglyceride breakdown (lipolysis) into fatty acids and glycerol (Lafontan 2008). Thus, we measured lipolysis and glucose uptake in AT (subcutaneous abdominal fat depot) from healthy subjects in response to increasing concentrations of apelin-13. The following results show that apelin stimulates AMPK and glucose uptake but had no effect on lipolysis in the conditions used.

Materials and methods

Subjects

The study was performed according to the Declaration of Helsinki, and human AT was collected according to the guidelines and approval of the Ethical Committee of Rangueil Hospital in Toulouse from healthy women undergoing abdominal dermolipectomy for plastic surgery (body mass index: 25·6±0·8 kg/m^2, age: 43·2±2·7 years, n=30 in total, each tissue was either attributed to one or several protocols). All of them were drug free and healthy. No clinical data from these patients were available.

Human AT preparation

Human subcutaneous AT was either digested by collagenase (type II, Sigma–Aldrich Co.) in order to get isolated adipocytes or minced into very small pieces (1–2 mg) to get explants. The explants were washed two times in PBS at 37 °C, centrifuged for 10 min at 850 ***g*** at room temperature in order to remove the connective tissue, and the medium was discarded. Isolated adipocytes were obtained after mincing AT in 5 ml of DMEM (Gibco, Invitrogen) supplemented with 1 mg/ml collagenase and 1% albumin (BSA) for 30 min at 37 °C under shaking. Digestion was followed by filtration through a 150 μm screen and the floating adipocytes were separated from the medium containing the stroma vascular fraction and washed twice in DMEM.

APJ mRNA expression in human AT

Total RNAs (1 μg) were isolated from either AT using RNeasy Lipid Tissue kits (Qiagen, Courtaboeuf, France) or isolated adipocytes using RNeasy kit (Qiagen). They were then reverse transcribed using random hexamers and Superscript II reverse transcriptase (Invitrogen). Real-time PCR was performed as previously described (Boucher *et al.* 2005). Briefly, real-time PCR was performed on 12·5 ng cDNA with both sense (hAPJ sense: GCCCTTGCTTTCTGAAAATCA) and antisense (hAPJ reverse: GGACAGTTAAAGGATGTGCATAGGA) oligonucleotides (Eurogentec, Angers, France) in a final volume of 20 μl using SYBR Green qPCR Master Mix (Eurogentec, Seraing, Belgium). Fluorescence was monitored and analyzed in a GeneAmp 7500 detection system instrument (Applied Biosystems, Warrington, UK). In parallel, analysis of the 18S ribosomal RNA was performed using the ribosomal RNA control Taqman Assay kit (Applied Biosystems) to normalize gene expression.

Immunohistochemical study of APJ

AT was fixed overnight in a paraformaldehyde 4% solution, then hydrated, and paraffin-embedded. AT sections were blocked with 1% BSA in Tris buffer for 1 h at room temperature. Sections were then incubated with APJ monoclonal (anti-human) antibody (R&D Systems, Lille, France) overnight at 4 °C (1:25 dilution). Control sections were stained with IgG mouse serum used at the same dilution as APJ antibody. As a secondary antibody, alkaline phosphatase (DakoCytomation, Trappes, France) was used at 1:100 dilution. Antigen visualization was achieved with an alkaline phosphate system (BCIP/NBT Substrate System) added with Levamisole (DakoCytomation) in order to suppress nonspecific staining.

Glucose uptake

AT explants were preincubated for 10 min in Krebs–Henseleit (KH) buffer, pH 7·4, containing BSA (2 mg/ml) and 20 mM Hepes. Explants were then incubated for 45 min in the presence or absence of different concentrations of [Pyr1]apelin-13 (BACHEM distribution services) or 100 nM insulin (Sigma–Aldrich

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Co.). [Pyr1]apelin-13 has been chosen as a stable biological active apelin isoform (Kleinz & Davenport 2005). In order to test the involvement of AMPK, C compound was added 20 min before apelin. For glucose transport, explants were transferred to another vial containing KH medium supplemented with 0·1 mM 2-deoxyglucose (2-DG) and D-[^{3}H]-2-DG (0·25 μCi/ml) for 10 min. All the incubations were carried out at 37 °C under a 95% O_2/5% CO_2 atmosphere. Explants were then washed two times in PBS under shaking and then lysed by using Precellys 24 automated biological sample lyser with CK-14 beads vials (Ozyme, Saint Quentin, France) in 500 μl NaOH (1 M). The infranatant was recovered for further extraction and 500 μl HCl (1 M) were added to each sample in order to restore pH neutrality. The total amount of D-[^{3}H]-2-DG was quantified after addition of a perchloric acid (6%) solution. In an other vial, D-[^{3}H]-2-DG 6-phosphate was precipitated by the use of zinc sulfate (0·3 M) and barium hydroxide (0·3 M). The amount of 2-DG internalized was calculated by the difference between the radioactivity found in total 2-DG and nonphosphorylated 2-DG found in the supernatants.

Glucose uptake by isolated adipocytes was measured as previously described (Iglesias-Osma *et al.* 2005). Briefly, adipocytes (1:10 dilution) were incubated with 1 μM of apelin or 100 nM insulin as a positive control for 45 min at 37 °C and 0·4 μCi 2-DG were added at a final concentration of 0·1 mM for 10 min. Assays were stopped with 100 μM cytochalasin B and aliquots of cell suspension were centrifuged in microtubes containing di-isononyl phthalate, which allowed separation of adipocytes from the buffer and counting of the radioactive intracellular 2-DG.

Lipolysis

Lipolysis was realized in Krebs–Ringer–Hepes (KRH) buffer with 2% BSA. In all, 100 μl AT microexplants or adipocytes (1:10 dilution) were incubated in 1 ml KRH for 1 h at 37 °C under gentle shaking in the presence of different concentrations of apelin or in 1 μM isoprenaline (a β-adrenergic agonist) as a positive control. The reaction was stopped once the tubes were on ice. Glycerol released in the medium was measured in a 30 μl aliquot using the Glycerol-Free Reagent kit (Sigma) while nonesterified fatty acids (NEFA) were measured in 15 μl of the medium by the WAKO NEFA kit (WAKO Chemicals, Montbonnot St Martin, France).

Western blots

AT explants were lysed as described above (Precellys) and loaded (50 μg protein per lane) on 10% SDS–PAGE gel and transferred to nitrocellulose membrane (Schleicher-Schuell). Membranes were blotted with anti-phospho-AMPKα-Thr-172 or anti-phospho-acetyl-CoA carboxylase (ACC)-Ser79 antibodies (Cell Signaling Technology, Beverly, MA, USA) used at 1:1000 dilution in Tris-buffered saline containing 5% BSA and Tween-20 at 0·01%. As a secondary antibody, anti-rabbit-HRP was used (1:3000 dilution). Membranes were probed with β-actin for total proteins. Immunoreactive proteins were detected using the ECL Plus (GE Healthcare, Orsay, France) and quantified by Image Quant TL software (GE Healthcare Bio-Sciences, Uppsala, Sweden).

Statistical analysis

Data are presented as means ± S.E.M. Analysis of differences between the groups was performed with one-way ANOVA followed by *post hoc* Bonferroni's or Dunnett's test, when appropriate and $P<0·05$ was considered to be significant.

Results

Expression of APJ receptors in human AT

We have previously shown that *APJ* mRNAs were present in human AT (Castan-Laurell *et al.* 2008, Dray *et al.* 2010). In this study, we further delineated the presence of both *APJ* mRNA and protein in isolated adipocytes compared to the entire AT. As shown in Fig. 1, *APJ* mRNA levels were lower in isolated adipocytes than in AT. APJ proteins were also visualized in human AT since a positive immunostaining was observed in the periphery of adipocytes compared to control.

Effect of apelin on AMPK

AMPK has been revealed as a target of apelin signaling in muscle (Dray *et al.* 2008, Yue *et al.* 2009), thus the effect of apelin on AMPK activation was studied in human AT. Time-course studies in AT explants revealed that 10 nM apelin induced the phosphorylation of AMPK that was maximal at 10 min (Fig. 2A). In total, 2 mM 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) incubated for 60 min was used as a positive control. Moreover, apelin stimulated, in parallel, the phosphorylation of AMPK and ACC, its downstream target enzyme, in a dose-dependent manner with a significant effect at 10 nM (Fig. 2B). Thus, apelin activates AMPK in human AT. Metabolic effects mediated by apelin involving AMPK were then studied.

Effect of apelin on lipolysis

Both glycerol and NEFA releases were measured as final products of triglyceride hydrolysis after 1 h incubation with different agents. Isoprenaline (a β-adrenergic

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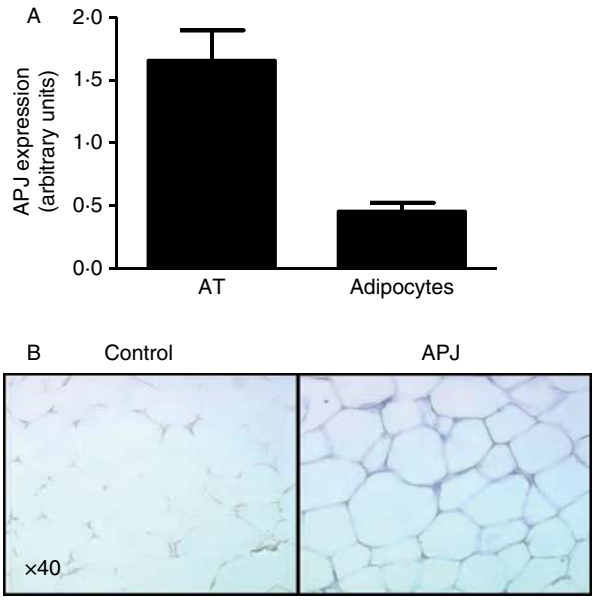

Figure 1 Expression of APJ, the apelin receptor, in human AT. (A) mRNA levels of APJ in entire adipose tissue (AT) and isolated adipocytes. Results are mean ± S.E.M. of eight independent experiments. (B) Representative immunostaining without (control) or with anti-APJ antibody (APJ) in human AT.

agonist) was used at 1 μM as a control of maximal lipolytic activation and AICAR was used at 2 mM as an activator of AMPK (Gaidhu *et al.* 2009). Isoprenaline stimulated the basal release of glycerol and NEFA from AT explants whereas AICAR decreased basal lipolysis (Fig. 3A). However, apelin, whatever the concentrations used, had no significant effect on basal and isoprenaline-stimulated lipolysis (Fig. 3A). In addition, in isolated adipocytes, apelin at 10 nM and 1 μM did not have significant effect on lipolysis. When added at the same time along with isoprenaline, apelin did not modify the response of the β-adrenergic agonist, while 100 nM insulin was antilipolytic (Fig. 3B).

Effect of apelin on glucose uptake

Insulin, as a positive control, stimulated glucose uptake in human AT explants (Fig. 4A). Apelin stimulated glucose uptake in a dose-dependent manner, with a significant effect at 10 nM, but to a lower extend compared to insulin (1·56-fold with 10 nM apelin versus 2·0-fold with 100 nM insulin). On isolated adipocytes, apelin had a weak effect (basal: 0·16 ± 0·04 pmol/g protein; apelin 1 μM: 0·23 ± 0·06 pmol/g protein, $n=7$–10). The involvement of AMPK in apelin-stimulated glucose transport was underlined by the use of C compound, a selective AMPK inhibitor. C compound (20 μM) prevented apelin-induced glucose uptake in human AT explants (Fig. 4B).

Discussion

In this study, we provide evidence that acute apelin treatment of human AT from healthy subjects stimulates AMPK phosphorylation and glucose uptake but has no effect on lipolysis. Thus, this study reveals, for the first time, an activation of AMPK by apelin in AT and

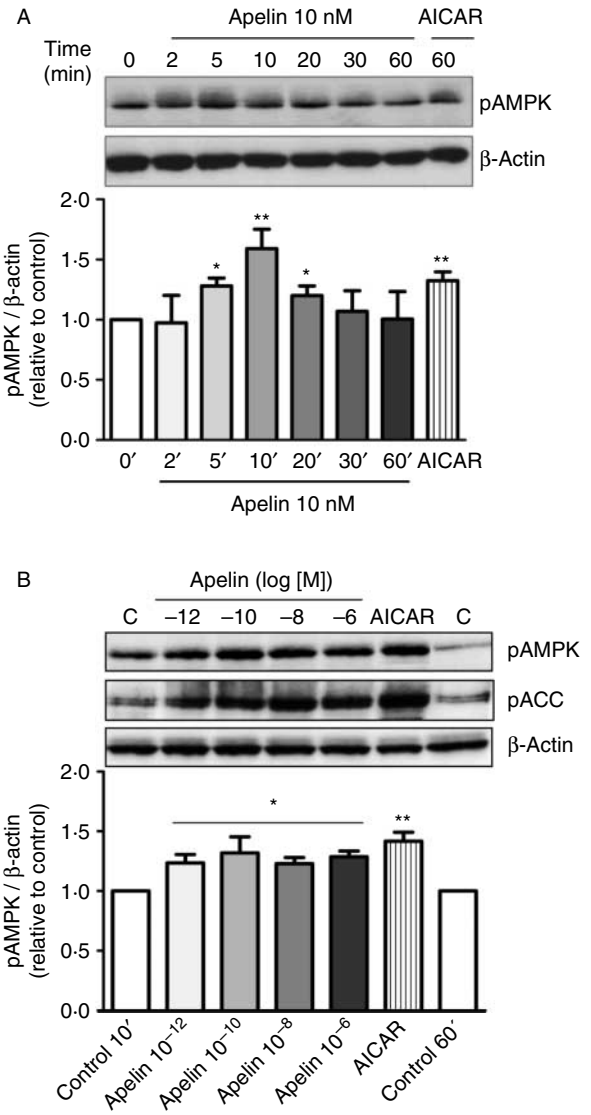

Figure 2 Apelin stimulates AMPK phosphorylation. (A) Representative blot of *in vitro* time-course study of Thr-172 AMPK phosphorylation in the presence of apelin (10 nM) or AICAR (2 mM), used as a positive control and incubated for 60 min. β-Actin was used to evaluate total proteins, $n=4$. The graph shows the quantified data, $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$ versus control. (B) Dose–response of apelin, at the indicated concentrations (incubation time: 10 min for apelin, 60 min for AICAR), on AMPK and ACC phosphorylation compared to control (C). β-Actin was used to evaluate total proteins. The graph shows the quantified data, $n=4$, $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$ versus control.

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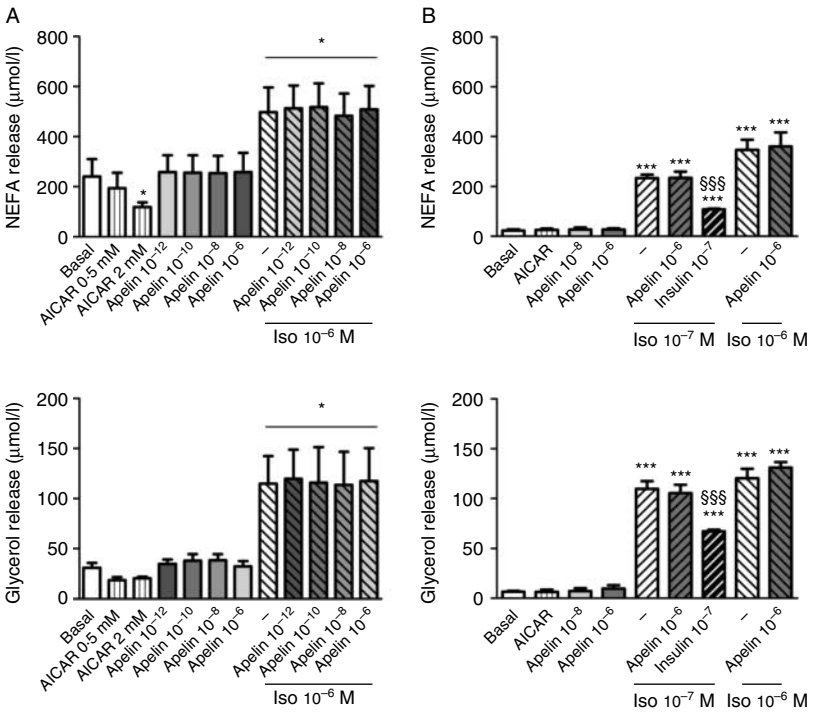

Figure 3 Effect of apelin on lipolysis in human AT explants (A) and isolated adipocytes (B). NEFA (top) and glycerol (bottom) releases upon basal lipolysis (white columns), under stimulation with AICAR alone, apelin alone or in the presence of isoprenaline (Iso, shaded columns) and insulin 100 nM in the presence of isoprenaline. Results are the mean ± S.E.M. of six independent experiments for AT explants and four independent experiments for isolated adipocytes. *$P<0{\cdot}05$ and ***$P<0{\cdot}001$ versus basal; §§§$P<0{\cdot}001$ for insulin + isoprenaline versus isoprenaline alone.

functional metabolic effects of apelin on humans. These results are in line with our previous study performed in mice indicating that i) *ex vivo*, in soleus skeletal muscle, apelin stimulated AMPK and glucose uptake (Dray *et al.* 2008) and that ii) *in vivo*, apelin perfusion during a euglycemic-hyperinsulinemic clamp stimulated glucose uptake in AT.

Lipolysis is one of the main metabolic functions of AT. An upstream step controlling this process is the intracellular levels of cAMP. Apelin has been shown to reduce forskolin-stimulated cAMP production in different cell lines overexpressing APJ receptors through a pertussis-toxin-sensitive G-protein (Masri *et al.* 2002, Bai *et al.* 2008). Thus, an antilipolytic effect of apelin was expected but no modification of basal or isoprenaline-stimulated lipolysis was observed in the conditions used. Moreover, apelin has been shown to activate AMPK in skeletal muscle (Dray *et al.* 2008) while AMPK also regulates energy metabolism in AT (Daval *et al.* 2006). However, depending on the studies, it has been shown that, in rodents, activation of AMPK could lead either to inhibition or stimulation of lipolysis (Yin *et al.* 2003, Hutchinson *et al.* 2005, Daval *et al.* 2006, Omar *et al.* 2009). Very recently, it was shown that protein kinase A, once activated by lipolytic agents, inactivates AMPK to promote efficient lipolysis (Djouder *et al.* 2010). AMPK activation is thus viewed as a consequence of ongoing re-esterification of fatty acids that consume energy (Gauthier *et al.* 2008, Djouder *et al.* 2010) and in fine AMPK activation restrains hydrolysis of triglycerides (lipolysis). Acute treatment (1 h) with AICAR has been shown to decrease both NEFA and glycerol release in rat adipocytes (Gaidhu *et al.* 2009). In this study, AICAR decreased both NEFA and glycerol release in agreement with the results reported by Gaidhu *et al.* (2009). Apelin incubated during the same period did not acutely inhibit lipolysis in basal conditions or during isoprenaline-stimulated lipolysis. This could be due to the less sustained effect of apelin on AMPK phosphorylation. These results are thus different from those obtained with biguanides and thiazolidinediones that activate AMPK and inhibit stimulated lipolysis in human adipocytes (Bourron *et al.* 2010). Although apelin shares with those antidiabetic drugs, a decrease in glycemia, and activation of AMPK, apelin might not act on acute lipolysis.

Among the other metabolic functions of AT involving AMPK, we focused on glucose uptake. In this study,

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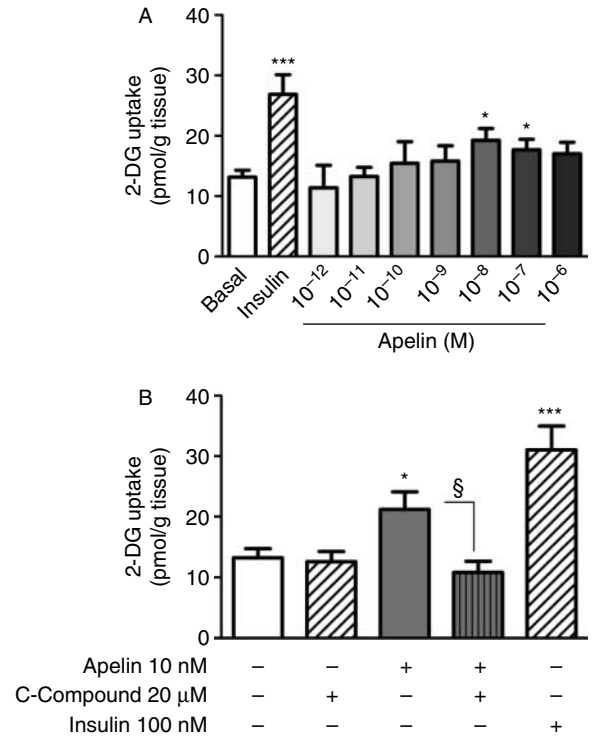

Figure 4 Apelin stimulates glucose uptake through AMPK activation. (A) AT explants were stimulated either with 100 nM insulin or with the indicated concentrations of apelin as indicated in the section 'Materials and methods'. Results are the mean ±S.E.M. of 7–12 independent experiments. *$P<0{\cdot}05$, ***$P<0{\cdot}001$ versus basal lipolysis (Basal). (B) Effect of 20 µM C compound on basal and apelin-stimulated glucose uptake in AT explants. Insulin was used as a positive control. Values are the mean±S.E.M., $n=7$. *$P<0{\cdot}05$, ***$P<0{\cdot}001$ versus basal. §$P<0{\cdot}05$ for apelin 10 nM versus apelin 10 nM+C compound.

apelin had a modest effect on glucose uptake in human AT explants and isolated adipocytes. Insulin, at physiological concentrations, stimulated glucose transport in human AT explants twofold above basal, which is in agreement with previous studies performed on human isolated adipocytes (Iglesias-Osma *et al.* 2005, Wanecq *et al.* 2009) or differentiated cultured adipocytes (Hauner *et al.* 1998). It should be noticed that conversion of glucose into fatty acids (*de novo* lipogenesis) especially in AT is much lower in humans compared to rodents (Zelewski & Swierczyński 1990). We choose to work first on AT explants with the aim to reproduce the conditions used for glucose transport *ex vivo* in muscle (Dray *et al.* 2008). It has been shown that functional activities are maintained in human AT explants (Moustaïd *et al.* 1996, Viguerie *et al.* 2002). Moreover, the effects of apelin were similar in AT explants and isolated adipocytes.

Only a few studies have demonstrated the role of AMPK in glucose uptake in adipose cells, and the mechanisms leading to AMPK activation in adipocytes remain poorly understood. The use of AICAR, as a pharmacological tool to activate this kinase, has not given conclusive results regarding its physiological role (Salt *et al.* 2000, Sakoda *et al.* 2002). Indeed, overexpression of a dominant negative form of AMPK in cultured adipocytes was shown to abolish AMPK activation without affecting AICAR-induced glucose transport (Sakoda *et al.* 2002). However, adiponectin, an insulin-sensitizing adipokine, was shown to increase glucose uptake in primary rat adipocytes through AMPK activation and this effect was abrogated in the presence of AMPK inhibitors (Wu *et al.* 2003). Similar results were obtained in this study with apelin. The effect of C compound, in blocking apelin-stimulated glucose uptake as well as the phosphorylation of ACC, strongly suggests a role of AMPK and thus an insulin-independent pathway for the effects of apelin. Yet, it is not known whether glucose uptake by adiponectin or apelin is dependent on glucose transporter-4 (GLUT4) translocation. GLUT4 is essential for insulin-stimulated glucose uptake in skeletal muscle and AT. Moreover, *GLUT4* mRNA levels in human AT are detected mostly in mature adipocytes compared to the stroma vascular fraction or nonadipocyte cells (Vitkova *et al.* 2007). Consequently, the GLUT4-dependent effects are mainly due to adipocyte response. Activation of AMPK in different cell types could lead to translocation of GLUT4 transporters (Mu *et al.* 2001, Li *et al.* 2004), but not in all the reports (Lemieux *et al.* 2003, Breen *et al.* 2008). Moreover, studies performed in cultured 3T3-L1 preadipocytes or in C2C12 myoblasts demonstrated that AMPK is also implicated in GLUT1-mediated glucose uptake (Abbud *et al.* 2000). Hence, apelin could activate AMPK and glucose uptake through different GLUTs depending on the cell type (preadipocytes or adipocytes) involved.

In conclusion, these results show that apelin stimulates glucose uptake in human AT but to a less extent than insulin. Given that apelin is produced and secreted by AT, a local action of apelin could affect differently AT metabolic functions compared to insulin action. It will be of interest to depict these effects in obese subjects. Moreover, we also described AMPK activation by apelin in AT. Since the activation of AMPK is viewed as a therapeutic approach in obesity-associated disorders (type 2 diabetes) and since only few compounds have been described to activate AMPK in this tissue, the apelin/APJ system could be a promising target with potential beneficial effects in humans.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Abbud W, Habinowski S, Zhang JZ, Kendrew J, Elkairi FS, Kemp BE, Witters LA & Ismail-Beigi F 2000 Stimulation of AMP-activated protein kinase (AMPK) is associated with enhancement of Glut1-mediated glucose transport. *Archives of Biochemistry and Biophysics* **380** 347–352. (doi:10.1006/abbi.2000.1935)

Bai B, Tang J, Liu H, Chen J, Li Y & Song W 2008 Apelin-13 induces ERK1/2 but not p38 MAPK activation through coupling of the human apelin receptor to the Gi2 pathway. *Acta Biochimica et Biophysica Sinica* **40** 311–318. (doi:10.1111/j.1745-7270.2008.00403.x)

Boucher J, Masri B, Daviaud D, Gesta S, Guigne C, Mazzucotelli A, Castan-Laurell I, Tack I, Knibiehler B, Carpene C *et al.* 2005 Apelin, a newly identified adipokine up-regulated by insulin and obesity. *Endocrinology* **146** 1764–1771. (doi:10.1210/en.2004-1427)

Bourron O, Daval M, Hainault I, Hajduch E, Servant JM, Gautier JF, Ferré P & Foufelle F 2010 Biguanides and thiazolidinediones inhibit stimulated lipolysis in human adipocytes through activation of AMP-activated protein kinase. *Diabetologia* **53** 768–778. (doi:10.1007/s00125-009-1639-6)

Breen DM, Sanli T, Giacca A & Tsiani E 2008 Stimulation of muscle cell glucose uptake by resveratrol through sirtuins and AMPK. *Biochemical and Biophysical Research Communications* **374** 117–122. (doi:10.1016/j.bbrc.2008.06.104)

Carpéné C, Dray C, Attané C, Valet P, Portillo MP, Churruca I, Milagro FI & Castan-Laurell I 2007 Expanding role for the apelin/APJ system in physiopathology. *Journal of Physiology and Biochemistry* **63** 358–373. (doi:10.1007/BF03165767)

Castan-Laurell I, Vitkova M, Daviaud D, Dray D, Kovacikova M, Kovacova Z, Hejnova J, Stich V & Valet P 2008 Effect of hypocaloric-induced weight loss in obese women on plasma apelin and adipose tissue expression of apelin and AP. *European Journal of Endocrinology* **158** 905–910. (doi:10.1530/EJE-08-0039)

Daval M, Foufelle F & Ferré P 2006 Functions of AMP-activated protein kinase in adipose tissue. *Journal of Physiology* **574** 55–62. (doi:10.1113/jphysiol.2006.111484)

Djouder N, Tuerk RD, Suter M, Salvioni P, Thali RF, Scholz R, Vaahtomeri K, Auchli Y, Rechsteiner H, Brunisholz RA *et al.* 2010 PKA phosphorylates and inactivates AMPKα to promote efficient lipolysis. *EMBO Journal* **29** 469–481. (doi:10.1038/emboj.2009.339)

Dray C, Knauf C, Daviaud D, Waget A, Boucher J, Buléon M, Cani PD, Attané C, Guigné C, Carpéné C *et al.* 2008 Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. *Cell Metabolism* **8** 437–445. (doi:10.1016/j.cmet.2008.10.003)

Dray C, Debard C, Jager J, Disse E, Daviaud D, Martin P, Attané C, Wanecq E, Guigné C, Bost F *et al.* 2010 Apelin and APJ regulation in adipose tissue and skeletal muscle of type 2 diabetic mice and humans. *American Journal of Physiology. Endocrinology and Metabolism* **298** E1161–E1169. (doi:10.1152/ajpendo.00598.2009)

Gaidhu MP, Fediuc S, Anthony NM, So M, Mirpourian M, Perry RL & Ceddia RB 2009 Prolonged AICAR-induced AMP-kinase activation promotes energy dissipation in white adipocytes: novel mechanisms integrating HSL and ATGL. *Journal of Lipid Research* **50** 704–715. (doi:10.1194/jlr.M800480-JLR200)

Gauthier MS, Miyoshi H, Souza SC, Cacicedo JM, Saha AK, Greenberg AS & Ruderman NB 2008 AMP-activated protein kinase is activated as a consequence of lipolysis in the adipocyte: potential mechanism and physiological relevance. *Journal of Biological Chemistry* **283** 16514–16524. (doi:10.1074/jbc.M708177200)

Hauner H, Röhrig K, Spelleken M, Liu LS & Eckel J 1998 Development of insulin-responsive glucose uptake and GLUT4 expression in differentiating human adipocyte precursor cells. *International Journal of Obesity and Related Metabolic Disorders* **22** 448–453. (doi:10.1038/sj.ijo.0800606)

Heinonen MV, Purhonen AK, Miettinen P, Paakkonen M, Pirinen E, Alhava E, Akerman K & Herzig KH 2005 Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. *Regulatory Peptides* **130** 7–13. (doi:10.1016/j.regpep.2005.05.003)

Heinonen MV, Laaksonen DE, Karhu T, Karhunen L, Laitinen T, Kainulainen S, Rissanen A, Niskanen L & Herzig KH 2009 Effect of diet-induced weight loss on plasma apelin and cytokine levels in individuals with the metabolic syndrome. *Nutrition, Metabolism, and Cardiovascular Diseases* **19** 626–633. (doi:10.1016/j.numecd.2008.12.008)

Higuchi K, Masaki T, Gotoh K, Chiba S, Katsuragi I, Tanaka K, Kakuma T & Yoshimatsu H 2007 Apelin, an APJ receptor ligand, regulates body adiposity and favors the messenger ribonucleic acid expression of uncoupling proteins in mice. *Endocrinology* **148** 2690–2697. (doi:10.1210/en.2006-1270)

Hutchinson DS, Chernogubova E, Dallner OS, Cannon B & Bengtsson T 2005 β-Adrenoceptors, but not α-adrenoceptors, stimulate AMP-activated protein kinase in brown adipocytes independently of uncoupling protein-1. *Diabetologia* **48** 2386–2395. (doi:10.1007/s00125-005-1936-7)

Iglesias-Osma MC, Bour S, Garcia-Barrado MJ, Visentin V, Pastor MF, Testar X, Marti L, Enrique-Tarancon G, Valet P, Moratinos J *et al.* 2005 Methylamine but not mafenide mimics insulin-like activity of the semicarbazide-sensitive amine oxidase-substrate benzylamine on glucose tolerance and on human adipocyte metabolism. *Pharmacology Research* **52** 475–484. (doi:10.1016/j.phrs.2005.07.008)

Kadoglou NP, Tsanikidis H, Kapelouzou A, Vrabas I, Vitta I, Karayannacos PE, Liapis CD & Sailer N 2010 Effects of rosiglitazone and metformin treatment on apelin, visfatin, and ghrelin levels in patients with type 2 diabetes mellitus. *Metabolism* **59** 373–379. (doi:10.1016/j.metabol.2009.08.005)

Kleinz MJ & Davenport AP 2005 Emerging roles of apelin in biology and medicine. *Pharmacology and Therapeutics* **107** 198–211. (doi:10.1016/j.pharmthera.2005.04.001)

Lafontan M 2008 Advances in adipose tissue metabolism. *International Journal of Obesity* **32** (Supplement 7) S39–S51. (doi:10.1038/ijo.2008.237)

Lemieux K, Konrad D, Klip A & Marette A 2003 The AMP-activated protein kinase activator AICAR does not induce GLUT4 translocation to transverse tubules but stimulates glucose uptake and p38 mitogen-activated protein kinases α and β in skeletal muscle. *FASEB Journal* **17** 1658–1665. (doi:10.1096/fj.02-1125com)

Li J, Hu X, Selvakumar P, Russell RR, Cushman SW, Holman GD & Young LH 2004 Role of the nitric oxide pathway in AMPK-mediated glucose uptake and GLUT4 translocation in heart muscle. *American Journal of Physiology. Endocrinology and Metabolism* **287** E834–E841. (doi:10.1152/ajpendo.00234.2004)

Li L, Yang G, Li Q, Tang Y, Yang M, Yang H & Li K 2006 Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. *Experimental and Clinical Endocrinology and Diabetes* **114** 544–548. (doi:10.1055/s-2006-948309)

Masri B, Lahlou H, Mazarguil H, Knibiehler B & Audigier Y 2002 Apelin (65–77) activates extracellular signal-regulated kinases via a PTX-sensitive G protein. *Biochemical and Biophysical Research Communications* **290** 539–545. (doi:10.1006/bbrc.2001.6230)

Moustaïd N, Jones BH & Taylor JW 1996 Insulin increases lipogenic enzyme activity in human adipocytes in primary culture. *Journal of Nutrition* **126** 865–870.

Mu J, Brozinick JT, Valladares O, Bucan M & Birnbaum MJ 2001 A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. *Molecular Cell* **7** 1085–1094. (doi:10.1016/S1097-2765(01)00251-9)

Omar B, Zmuda-Trzebiatowska E, Manganiello V, Göransson O & Degerman E 2009 Regulation of AMP-activated protein kinase by

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cAMP in adipocytes: roles for phosphodiesterases, protein kinase B, protein kinase A, Epac and lipolysis. *Cellular Signalling* **21** 760–766. (doi:10.1016/j.cellsig.2009.01.015)

Sakoda H, Ogihara T, Anai M, Fujishiro M, Ono H, Onishi Y, Katagiri H, Abe M, Fukushima Y, Shojima N *et al.* 2002 Activation of AMPK is essential for AICAR-induced glucose uptake by skeletal muscle but not adipocytes. *American Journal of Physiology. Endocrinology and Metabolism* **282** E1239–E1244. (doi:10.1152/ajpendo.00455.2001)

Salt IP, Connell JM & Gould GW 2000 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR) inhibits insulin-stimulated glucose transport in 3T3-L1 adipocytes. *Diabetes* **49** 1649–1656. (doi:10.2337/diabetes.49.10.1649)

Soriguer F, Garrido-Sanchez L, Garcia-Serrano S, Garcia-Almeida JM, Garcia-Arnes J, Tinahones FJ & Garcia-Fuentes E 2009 Apelin levels are increased in morbidly obese subjects with type 2 diabetes mellitus. *Obesity Surgery* **19** 1574–1580. (doi:10.1007/s11695-009-9955-y)

Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C *et al.* 1998 Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochemical and Biophysical Research Communications* **251** 471–476. (doi:10.1006/bbrc.1998.9489)

Viguerie N, Millet L, Avizou S, Vidal H, Larrouy D & Langin D 2002 Regulation of human adipocyte gene expression by thyroid hormone. *Journal of Clinical Endocrinology and Metabolism* **87** 630–634. (doi:10.1210/jc.87.2.630)

Vitkova M, Klimcakova E, Kovacikova M, Valle C, Moro C, Polak J, Hanacek J, Capel F, Viguerie N, Richterova B *et al.* 2007 Plasma levels and adipose tissue messenger ribonucleic acid expression of retinol-binding protein 4 are reduced during calorie restriction in obese subjects but are not related to diet-induced changes in insulin sensitivity. *Journal of Clinical Endocrinology and Metabolism* **92** 2330–2335. (doi:10.1210/jc.2006-2668)

Wanecq E, Prévot D & Carpéné C 2009 Lack of direct insulin-like action of visfatin/Nampt/PBEF1 in human adipocytes. *Journal of Physiology and Biochemistry* **65** 351–360. (doi:10.1007/BF03185930)

Wu X, Motoshima H, Mahadev K, Stalker TJ, Scalia R & Goldstein BJ 2003 Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. *Diabetes* **52** 1355–1363. (doi:10.2337/diabetes.52.6.1355)

Yin W, Mu J & Birnbaum MJ 2003 Role of AMP-activated protein kinase in cyclic AMP-dependent lipolysis in 3T3-L1 adipocytes. *Journal of Biological Chemistry* **278** 43074–43080. (doi:10.1074/jbc.M308484200)

Yue P, Jin H, Aillaud-Manzanera M, Deng AC, Azuma J, Asagami T, Kundu RK, Reaven GM, Quertermous T & Tsao PS 2009 Apelin is necessary for the maintenance of insulin sensitivity. *American Journal of Physiology. Endocrinology and Metabolism* **298** E59–E67. (doi:10.1152/ajpendo.00385.2009)

Zelewski M & Swierczyński J 1990 Comparative studies on lipogenic enzyme activities in the liver of human and some animal species. *Comparative Biochemistry and Physiology* **95** 469–472. (doi:10.1016/0305-0491(90)90004-D)

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