

The dietary flavonoids naringenin and quercetin acutely impair glucose metabolism in rodents possibly via inhibition of hypothalamic insulin signalling

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Abstract

Secondary metabolites of herbs and spices are widely used as an alternative strategy in the therapy of various diseases. The polyphenols naringenin, quercetin and curcumin have been characterised as anti-diabetic agents. Conversely, *in vitro*, naringenin and quercetin are described to inhibit phosphoinositide-3-kinase (PI3K), an enzyme that is essential for the neuronal control of whole body glucose homeostasis. Using both *in vitro* and *in vivo* experiments, we tested whether the inhibitory effect on PI3K occurs in neurons and if it might affect whole body glucose homeostasis. Quercetin was found to inhibit basal and insulin-induced phosphorylation of Akt (Ser473), a downstream target of PI3K, in HT-22 cells, whereas naringenin and curcumin had no effect. In Djungarian hamsters (*Phodopus sungorus*) naringenin and quercetin (10 mg/kg administered orally) diminished insulin-induced phosphorylation of Akt (Ser473) in the arcuate nucleus, indicating a reduction in hypothalamic PI3K activity. In agreement with this finding, glucose tolerance in naringenin-treated hamsters (oral) and mice (oral and intracerebroventricular) was reduced compared with controls. Dietary quercetin also impaired glucose tolerance, whereas curcumin was ineffective. Circulating levels of insulin and insulin-like growth factor-binding protein were not affected by the polyphenols. Oral quercetin reduced the respiratory quotient, suggesting that glucose utilisation was impaired after treatment. These data demonstrate that low doses of naringenin and quercetin acutely and potently impair glucose homeostasis. This effect may be mediated by inhibition of hypothalamic PI3K signalling. Whether chronic impairments in glucose homeostasis occur after long-term application remains to be identified.

Key words: Phosphoinositide-3-kinase/insulin receptor substrate signalling; Arcuate nucleus; Glucose homeostasis

Herbs and spices have become increasingly popular for the therapy of many diseases. Secondary metabolites of herbs and spices, especially flavonoids and bioactive polyphenols, have been reported to be anti-oxidative, immunomodulating, anti-carcinogenic, anti-allergic, anti-inflammatory and anti-diabetic⁽¹⁾, exerting their biological effects via the modulation of enzymatic activity or free-radical scavenging⁽²⁾.

The polyphenols naringenin, quercetin and curcumin are common secondary metabolites. Curcumin, a curcuminoid, is used for food colouring (E100) and as a flavouring agent. It naturally occurs in the spice turmeric (*Curcuma longa*) and other curcuma species^(3,4). Naringenin and quercetin belong to the family of flavonoids, an important group of secondary metabolites with more than 5000 naturally occurring

metabolites⁽²⁾. Naringenin is the aglycone of naringin, which is found in citrus fruits, especially in grapefruits⁽⁵⁾, where it contributes to the colour and the bitter flavour. A large variety of fruits and vegetables are sources of quercetin, including black and green tea (*Camellia sinensis*) and onions^(6,7). All three polyphenols have been described to exhibit anti-diabetic properties^(8–11). In contrast, naringenin and quercetin inhibit insulin signalling *in vitro*. The maintenance of whole body glucose homeostasis is mediated via insulin stimulation of the phosphoinositide-3-kinase (PI3K) pathway, resulting in phosphorylation of the downstream target Akt (also known as protein kinase B^(12,13)). Interestingly, naringenin and quercetin show structural similarities to the synthetic PI3K inhibitors LY294002⁽¹⁴⁾ and TGX-221 (Fig. 1) and inhibit

Abbreviations: ARC, arcuate nucleus; AUC, area under the curve; ICV, intracerebroventricular; IGFBP2, insulin-like growth factor-binding protein 2; ip, intra-peritoneal; LD, long day; pAkt, phospho-Akt; PI3K, phosphoinositide-3-kinase; RQ, respiratory quotient; SD, short day.

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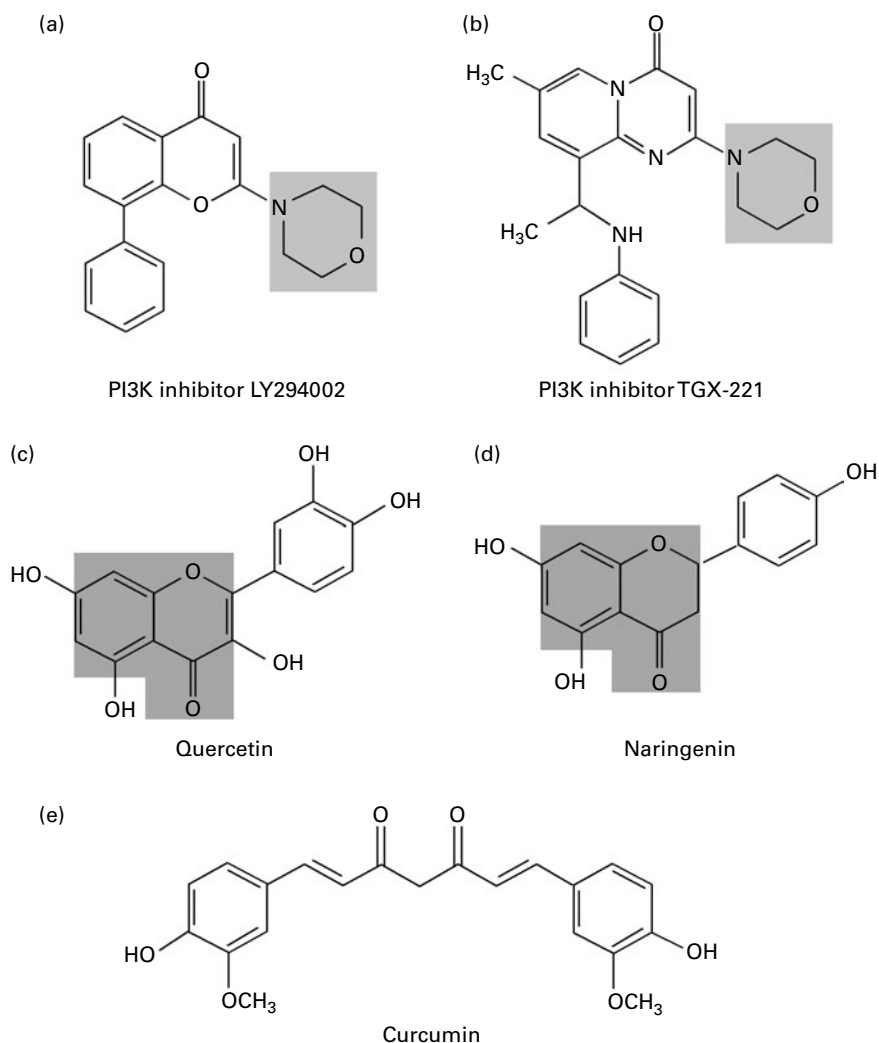


Fig. 1. Molecular structure of the phosphoinositide-3-kinase (PI3K) inhibitors (a) LY294002 and (b) TGX-221 and the polyphenols (c) quercetin, (d) naringenin and (e) curcumin. The morpholino ring of the PI3K inhibitors, which mediates the inhibitory effect on PI3K, is highlighted in light grey. The flavonoids quercetin and naringenin exhibit a related structure, the chromone moiety, marked in grey. It has been described that the chromone moiety of quercetin imitates the morpholino ring and blocks PI3K in a similar manner to the synthetic PI3K inhibitors. Curcumin offers no related structure.

PI3K *in vitro*^(14,15). In contrast, curcumin has been reported to activate PI3K⁽¹⁶⁾ and its chemical structure differs from that of naringenin and quercetin.

Our knowledge of the inhibition of PI3K by naringenin and quercetin is restricted to studies on cell lines of peripheral origin. It had been, however, well established that insulin signalling via insulin receptor substrate/PI3K in the hypothalamus is the key regulator of whole body glucose homeostasis^(12,17,18). Therefore, the focus of the present study is the characterisation of acute effects of these polyphenols on central insulin signalling and its contribution to whole body glucose homeostasis in both *in vitro* and *in vivo* experiments. Using neuronal cells, we analysed whether the polyphenols affect insulin signalling. We also assessed whether flavonoids affect PI3K signalling in the brain, together with whole body glucose homeostasis and glucose utilisation. These experiments were carried out in the Djungarian hamster (*Phodopus sungorus*, also known as

the Siberian hamster), a well-established animal model for the studies of metabolic impairments, and in mice. The hamster reveals pronounced seasonal changes in its physiology and neuroendocrinology⁽¹⁹⁾. During a summer-like photoperiod (long day (LD)), *P. sungorus* increases its body weight, whereas in a winter-like photoperiod (short day (SD)), the hamster loses body weight.

Material and methods

Cell culture

Mouse hippocampal neuronal cells (HT-22) were used to investigate the effect of naringenin (CarlRoth), quercetin (Sigma-Aldrich) and curcumin (Sigma-Aldrich) on insulin signalling. Cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% ciprofloxacin and 0.5% non-essential amino acids at an

atmosphere of 16% O₂, 79% N₂ and 5% CO₂ (by vol.). Cells were replenished with fresh medium before treatment with polyphenols in increasing concentrations of 25, 50, 75 and 100 μM for 45 min followed by insulin (25 nM) stimulation for another 45 min to measure phospho-Akt (pAkt) (Ser473). The experiment was repeated three times for the validation of the results.

Protein isolation and Western blot

Total cell lysates were isolated using Ripa lysis buffer (50 mM-Tris-Cl, 150 mM-NaCl, 1 mM-EDTA, 1% NP-40 and 0.25% sodium deoxycholate) including phosphatase inhibitors (1 mM-Na₃VO₄, 20 mM-NaF) and a protease inhibitor tablet (Roche). The protein content in the cell lysates was estimated using a bicinchoninic acid assay kit (Thermo Fisher Scientific, Inc.). A measure of 30 μg of the total protein was used for Western blot analysis. The pAkt and total Akt levels were detected using antibodies (1:1000) against pAkt (Ser473) and total Akt (Cell Signaling Technology, Inc.).

Animals

Adult female Djungarian hamsters were bred and maintained under LD conditions (16 h light–8 h dark) in the animal facility of the University of Marburg. After weaning, one group remained in LD and the other group was transferred to SD conditions with a light–dark cycle of 8–16 h. All hamsters were kept individually at an ambient temperature of 23°C and were 2–4 months old at the time of the experiments. SD hamsters were kept under SD conditions for 2 months until they were fully adapted to the short photoperiod.

Female C57BL/6J mice (8 week old) were purchased from Janvier. They were housed individually with a light–dark cycle of 12–12 h and at a room temperature of 26°C.

All animals had access to food and water *ad libitum*, unless otherwise stated. To determine the respiratory quotient (RQ), hamsters were food restricted 8 h before quercetin application. In all other experiments, hamsters and mice were food deprived for 16 h prior to the experiments.

All procedures were in accordance with the Guidelines of the German Council of Animal Care.

Polyphenol administration

Naringenin, quercetin and curcumin were dissolved in 10% ethanol–water and were administered orally. For the oral application, animals were anaesthetised with ketamine (hamster: 70 mg/kg body weight; mouse: 40 mg/kg body weight; Essex Tierarznei Niederlassung), an anaesthetic that does not affect glucose metabolism⁽²⁰⁾. Hamsters employed in the metabolic measurements were anaesthetised for a maximum of 30 s with 4% isoflurane (CP-Pharma Handelsgesellschaft) for oral application. Intracerebroventricular (ICV) injection of naringenin (2 μl; 5 nmol in 5% dimethyl sulphoxide/artificial cerebrospinal fluid) in mice via stereotaxically implanted cannulae⁽²¹⁾ was performed without any anaesthesia.

Anaesthetics and their influence on basal blood glucose concentration

To exclude the possibility that the use of anaesthetics might distort the effects of polyphenols on glucose homeostasis, we tested whether ketamine or very brief isoflurane (maximal 30 s) anaesthesia alters basal blood glucose levels in hamsters. LD female hamsters were fasted for 16 h and divided into three groups. One group was anaesthetised with ketamine (70 mg/kg body weight; *n* 7), the second group was briefly anaesthetised with isoflurane (4% for maximum of 30 s; *n* 5) and the third group was decapitated without anaesthesia (*n* 9); blood glucose concentrations were detected by using a commercially available glucometer (Accu-Check Performa; Roche). To determine the blood glucose levels of anaesthetised hamsters, the *vena sublingualis* was punctured and the glucose concentration was measured.

Insulin-induced phosphorylation of Akt (Ser473)

PI3K activity was analysed by detecting the phosphorylation of Akt (Ser473) after polyphenol treatment and insulin injection, as described previously^(21,22). pAkt (Ser473) immunoreactive cells in the arcuate nucleus (ARC) were counted by two investigators blind to the treatments. The total insulin-induced activation of PI3K without polyphenol pretreatment is represented as 100% pAkt (Ser473) counted cells; all other values are represented relative to this value in percentage.

Glucose tolerance tests after polyphenol treatment

The effect of oral naringenin on peripheral glucose tolerance was analysed in LD and SD hamsters (LD: *n* 3–8 hamsters/group; SD: *n* 5 hamsters/group). In mice, the effect of oral (*n* 8 mice/group) as well as central (*n* 3 mice/group) naringenin was assessed. The oral effect of curcumin and quercetin on glucose tolerance was determined in LD hamsters only (*n* 16–20 hamsters/group).

Fasted (16 h) animals were treated orally or centrally with either polyphenol (oral: 10 mg/kg body weight; ICV: 5 nmol/mouse) or vehicle (oral: 10% ethanol–water; ICV: 5% dimethyl sulphoxide/artificial cerebrospinal fluid). At 15 min after polyphenol treatment, the animals received an intraperitoneal (ip) glucose injection (hamster: 2 g/kg body weight; mouse: 1 g/kg body weight).

To determine blood glucose concentrations, the *vena sublingualis* (hamster) or the *vena facialis* (mouse) was punctured and the glucose concentration was detected⁽²¹⁾. Due to restricted blood withdrawal from the *vena sublingualis*, hamsters were anaesthetised with ketamine during the entire glucose tolerance test. Mice were anaesthetised (ketamine) for oral naringenin application only.

For central application, steel cannulae were stereotaxically implanted into the lateral cerebral ventricle of mice. The details of the procedure have been described previously⁽²¹⁾.

Insulin and insulin-like growth factor-binding protein 2 ELISA

To test potential effects of the polyphenols on circulating levels of insulin and insulin-like growth factor-binding protein 2 (IGFBP2), we performed an ELISA. To detect the insulin concentration in blood serum, food-restricted hamsters were pre-treated orally with naringenin, quercetin, curcumin or vehicle (n 6 hamsters/group). At 15 min after polyphenol treatment, insulin production and secretion were stimulated via an ip glucose injection (2 g/kg body weight). The *vena sublingualis* was punctured 30 min later and blood was collected using uncoated capillary tubes and prepared for insulin measurement. To detect serum IGFBP2 levels, fasted hamsters were treated orally with the polyphenols or vehicle (10% ethanol–water). At 60 min after treatment, blood was collected and stored on ice for approximately 1.5 h and then centrifuged for 20 min at 3500 rpm at 4°C. The supernatant containing serum was used to measure the serum insulin (Linco Research) and serum IGFBP2 concentrations (Alpco) by ELISA.

Metabolic measurement

To measure metabolism, VCO_2 and VO_2 were measured in hamsters in metabolism cages (approximately 1.8 litres; 17 cm × 11 cm × 12 cm). Measurements were taken in the morning at a constant ambient temperature of 23°C. The air flow in the cage was adjusted to approximately 45 l/h and continuously monitored. The procedure has been described in detail previously⁽²³⁾.

Individually caged hamsters had no access to food, resulting in a slow decrease in RQ. After approximately 8 h, the RQ had gone down to approximately 0.85; the hamsters were taken out of the chamber and anaesthetised briefly using isoflurane. Quercetin (10 mg/kg body weight) or vehicle (10% ethanol–water) was administered orally; 15 min later, hamsters that recovered from anaesthesia received an ip injection of glucose (2 g/kg body weight) or vehicle (NaCl) and were retransferred to the climate chamber. The RQ was measured for the next 90 min (n 10 hamsters/group). For consistent results, only hamsters with an RQ of approximately 0.85 were used in the experiment.

Body weight gain, food intake and water consumption after polyphenol administration

To analyse the effect of the polyphenols on energy metabolism, LD hamsters were food restricted for 24 h. At 1 h before the onset of the dark phase, hamsters were orally treated with quercetin, naringenin, curcumin (10 mg/kg; n 9/group) or vehicle (10% ethanol–water; n 8). Body weight changes, food intake and water consumption were measured 4 and 24 h after oral polyphenol administration.

Statistics

In vitro data were analysed by two-way ANOVA followed by the Holm–Sidak comparison test, using SigmaStat statistical software (Jandel).

Animal data were analysed by one-way ANOVA followed by the Holm–Sidak comparison test (SigmaStat), as appropriate. Where data failed equal variance or normality tests, they were analysed by one-way ANOVA followed by Dunn's multiple comparison test. The hamster naringenin glucose tolerance test was analysed by two-way ANOVA followed by the Holm–Sidak comparison test. Results are presented as means with their standard errors and $P < 0.05$ was considered significant.

Results

Effect of polyphenols on Ser473 phosphorylation of Akt in HT-22 cells

Mouse hippocampal neuronal cells (HT-22) were treated with increasing concentrations of naringenin, quercetin and curcumin to investigate the effect of these compounds on phosphorylation of Akt (Ser473). Cells treated with 25 nM-insulin for 45 min significantly increased the amount of pAkt (Ser473) protein by greater than 2-fold (vehicle *v.* insulin; $P < 0.001$). Naringenin-treated cells failed to inhibit both basal as well as insulin-stimulated phosphorylation of Akt (Ser473) at all concentrations (Fig. 2(a)). Quercetin potently inhibited basal phosphorylation of Akt at all four concentrations (vehicle/vehicle *v.* quercetin/vehicle; $P = 0.02$). Insulin-stimulated phosphorylation was inhibited by quercetin (vehicle/insulin *v.* quercetin/insulin; $P = 0.009$), reducing pAkt to an almost basal level (Fig. 2(b)). As with naringenin, curcumin treatment failed to inhibit both basal and insulin-stimulated phosphorylation of Akt (Ser473) at lower concentrations. However, pAkt (Ser473) levels were undetectable when curcumin was given at a high concentration of 100 μ M (Fig. 2(c)). Polyphenol treatment only affected the phosphorylation of Akt, while total Akt was not influenced by polyphenols, indicating viability of the cells.

Effect of polyphenols on insulin-induced phosphorylation of Akt (Ser473) in the arcuate nucleus

To investigate whether these polyphenols show effects on PI3K *in vivo*, we characterised the acute effect of polyphenol treatment on hypothalamic PI3K signalling in hamsters by measuring pAkt (Ser473) immunoreactive cells in the hypothalamus. Hamsters were treated orally with naringenin, quercetin, curcumin or vehicle followed by an ip insulin injection 15 min later. After 15 min, the hamsters were killed. We recently have established that insulin induces a robust increase in the number of pAkt (Ser473) immunoreactive cells in the ARC^(21,22). Thus, the number of cells counted in animals treated with insulin alone was considered to represent maximal stimulation, i.e. 100% (n 10 hamsters). Oral pre-treatment with the flavonoids naringenin (n 8 hamsters) and quercetin (n 6 hamsters) reduced the number of insulin-induced pAkt (Ser473) cells in the ARC by approximately 20% (Fig. 3(a, b)), suggesting an inhibition of insulin stimulation (vehicle *v.* naringenin; $P = 0.016$; vehicle *v.* quercetin; $P = 0.049$). In contrast, curcumin pre-treatment did not alter the number

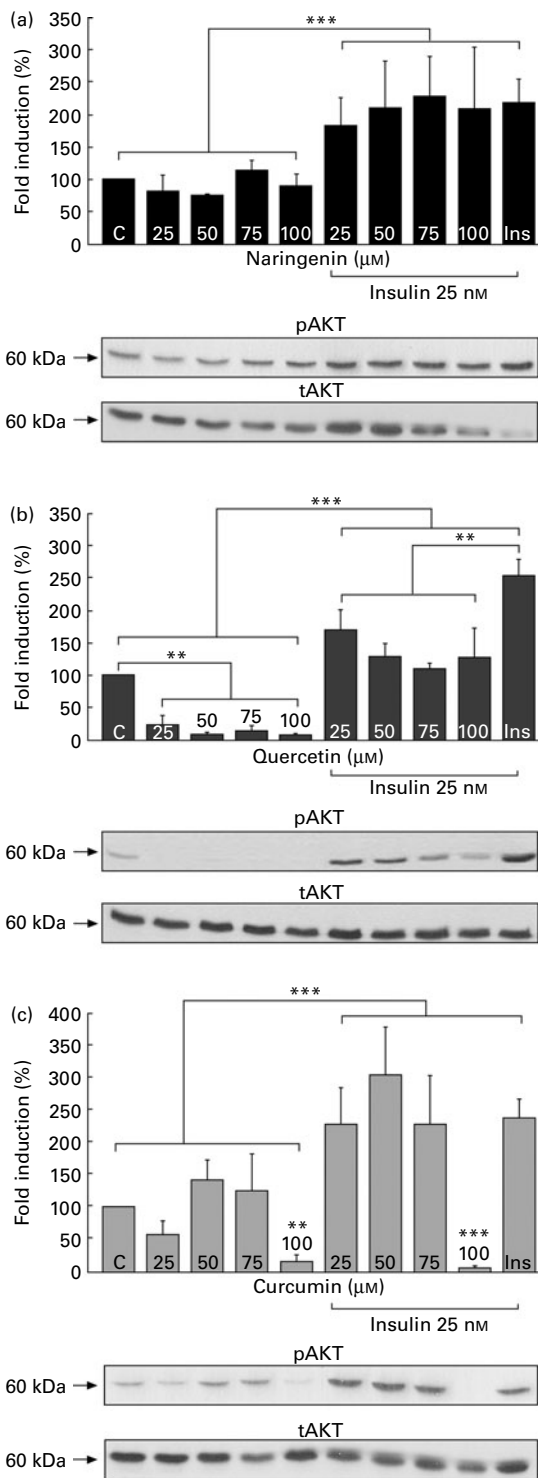


Fig. 2. Quercetin significantly reduced insulin (Ins)-induced phospho-Akt (pAkt) (Ser473) in HT-22 cells. Mouse hippocampal cells (HT-22) were treated with vehicle or the respective polyphenol in increasing concentrations from 25 to 100 μM . (a) Naringenin; (b) quercetin; (c) curcumin with or without Ins (25 mM) stimulation. The ratio between pAkt and total Akt was semi-quantitatively assessed by immunoblotting using a phosphor-specific antibody for pAkt. One of three quantified Western blot membranes representing pAkt (Ser473) and total Akt protein is shown below the bar charts. C, control. Values are means with their standard errors. Mean values were significantly different: ** $P < 0.01$, *** $P < 0.001$.

of insulin-induced pAkt immunoreactive cells in the ARC (Fig. 3(a, b); n 8 hamsters). In brain regions outside the hypothalamus, pAkt immunoreactive cells were not detectable (data not shown).

Effect of polyphenols on peripheral glucose tolerance

Having established that naringenin and quercetin inhibit neuronal insulin signalling, we investigated whether naringenin and quercetin could also affect glucose homeostasis in the animals.

Due to the limitation of blood withdrawal from *vena sublingualis* in hamsters under anaesthesia, we first tested whether ketamine anaesthesia *per se* affected basal blood glucose levels. Basal blood glucose levels of decapitated non-anaesthetised and ketamine anaesthetised hamsters were not significantly different. They were in a range of 700–750 mg/l (hamsters without anaesthesia = 752 (SEM 4.3) mg/l (n 7), anaesthetised hamsters = 704 (SEM 8.5) mg/l (n 9)), confirming that ketamine anaesthesia has no effect on glucose levels. This is consistent with other rodent species⁽²⁰⁾.

Firstly, we analysed the acute effect of an oral naringenin application on peripheral glucose tolerance in LD (n 3–8 hamsters/group) and SD acclimated hamsters (n 5–8 hamsters/group). Independent of the photoperiod, naringenin impaired glucose tolerance profoundly (Fig. 4(a)). The area under the curve (AUC) of naringenin-treated animals was significantly increased (naringenin *v.* vehicle, $P < 0.001$; LD naringenin *v.* vehicle, $P = 0.002$; SD naringenin *v.* vehicle, $P = 0.016$). No interaction between photoperiod and treatment occurred. To exclude the possibility that the observed phenomenon is restricted to hamsters only, we also performed an intra-peritoneal glucose tolerance test in C57BL/6J mice. As in the hamster, orally administered naringenin impaired whole body glucose tolerance in mice (n 8 mice/group, Fig. 4(b)) with a significant increase in AUC after this treatment (naringenin *v.* vehicle, $P = 0.01$).

To ascertain whether this impairment of whole body glucose homeostasis by flavonoid treatment is mediated via inhibition of PI3K in the brain, we administered naringenin ICV (n 3 mice/group). The flavonoid was as effective in impairing whole body glucose tolerance (naringenin *v.* vehicle; $P = 0.004$) as when given peripherally (Fig. 4(c)). As naringenin impaired glucose homeostasis robustly and independently of photoperiod or species, all subsequent experiments were carried out in LD hamsters only. In LD hamsters, quercetin also impaired glucose tolerance at a similar magnitude as naringenin (n 16–20 hamsters/group). The AUC was significantly increased compared with vehicle-treated animals (Fig. 4(d); quercetin *v.* vehicle, $P < 0.001$). However, consistent with the data shown above, curcumin (n 16 hamsters) had no effect on peripheral glucose tolerance (Fig. 4(d)).

Effect of polyphenol treatment on blood serum parameters

In the present experiment, we investigated whether polyphenols caused any alteration in circulating levels of,

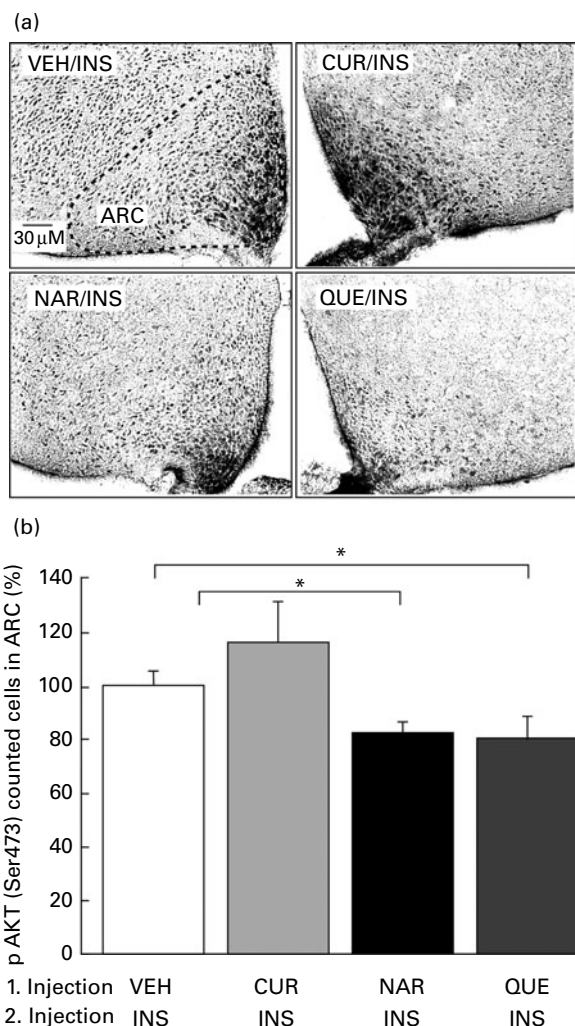


Fig. 3. Naringenin (NAR) and quercetin (QUE) decreased insulin (INS)-induced phospho-Akt (pAkt) (Ser473) in the arcuate nucleus (ARC) of Djungarian hamsters. Immunohistochemistry for pAkt (Ser473) was performed on brain sections of Djungarian hamsters. Long day hamsters received vehicle (VEH), NAR, QUE or curcumin (CUR) (10 mg/kg body weight administered orally) 30 min before intra-peritoneal INS injection. At 15 min after INS injection, hamsters were transcardially perfused. (a) Representative images showing pAkt (Ser473) immunoreactivity in the ARC of Djungarian hamsters. (b) The bar graph shows the number of immunoreactive pAkt (Ser473) cells in the ARC (n 6–10 hamsters/group). Mean value of the control was defined as 100%, and all other values are expressed in percentage to this value. Values are means with their standard errors. Mean values were significantly different: * P < 0.05.

e.g. serum insulin and serum IGF2BP2. The latter has recently been shown to contribute to the insulin-sensitising action of central leptin⁽²⁴⁾. Hamsters were treated orally with polyphenols prior to a glucose tolerance test (2 g/kg body weight). The glucose-stimulated insulin secretion after all polyphenol pre-treatments was similar to the vehicle-injected group (n 6 hamsters/group), with insulin concentrations in the range of 3–4 ng/ml (Fig. 5(a)). Also, the polyphenols had no effect on IGF2BP2 concentration, which was in the range of approximately 120–130 ng/ml (Fig. 5(b); n 6 hamsters/group) for all animals.

Effect of quercetin on glucose metabolism

At the doses tested, quercetin was the most potent polyphenol at impairing glucose tolerance. Therefore, we investigated whether quercetin affects glucose metabolism in hamsters. We analysed whether dietary quercetin influences the RQ as a marker for metabolic adjustments. As in previous experiments⁽²⁵⁾, a typical RQ of approximately 0.9 was seen in hamsters, which decreased during the period of food deprivation to approximately 0.85. Increased glucose utilisation, as reflected in an increase in the RQ, was typically achieved after an ip glucose injection (2 g/kg body weight). Quercetin pre-treatment 15 min before glucose application reduced glucose utilisation, shown by an alleviated glucose-stimulated increase of the RQ in quercetin pre-treated animals (n 10 hamsters/group; Fig. 6(a)). The maximal rise in the RQ was decreased in quercetin pre-treated hamsters compared with vehicle pre-treated hamsters, while the oxygen consumption was identical in both groups (Fig. 6(d)). The glucose-induced rise in RQ, defined as Δ RQ, was significantly reduced after quercetin pre-treatment (Fig. 6(b); P = 0.006). Additionally, the incremental AUC of quercetin pre-treated hamsters was decreased (Fig. 6(c); quercetin *v.* vehicle, P = 0.03). For evaluation of incremental AUC, the time interval from 0 (quercetin and glucose application) up to 60 min was used (light grey highlighted in Fig. 6(a)).

The very brief isoflurane anaesthesia (maximal 30 s), which was necessary for oral application of quercetin, had no effect on basal blood glucose concentration. Basal blood glucose concentrations of anaesthetised and non-anaesthetised hamsters were comparable and in a range of 710–750 mg/l (non-anaesthetised hamsters = 752 (SEM 4.3) mg/l (n 7); anaesthetised hamsters = 712 (SEM 2.5) mg/l (n 5)).

Body weight gain, food intake and water consumption after polyphenol administration

We next analysed whether polyphenols modulate energy metabolism of LD hamsters. Body weight gain of hamsters treated with quercetin or naringenin was significant at 4 and 24 h after administration compared with controls (quercetin *v.* vehicle, P = 0.0103; naringenin *v.* vehicle, P = 0.021; Fig. 7(a); n 8–9/group). Food intake of quercetin- and naringenin-treated hamsters was increased 24 h after flavonoid administration (quercetin *v.* vehicle, P = 0.01; naringenin *v.* vehicle, P = 0.024; Fig. 7(b); n 8–9/group). Consistent with the above experiments, curcumin also did not affect energy metabolism of hamsters. Body weight gain and food intake of curcumin-treated hamsters was comparable with controls (Fig. 7(a, b); n 8–9/group). Water consumption was not influenced by treatment with any polyphenol (Fig. 7(c); n 8–9/group).

Discussion

Whether the polyphenols quercetin, naringenin and curcumin exert anti- or pro-diabetic properties has not been well characterised. According to previous *in vitro* and *in vivo* experiments, their actions on glucose metabolism remain

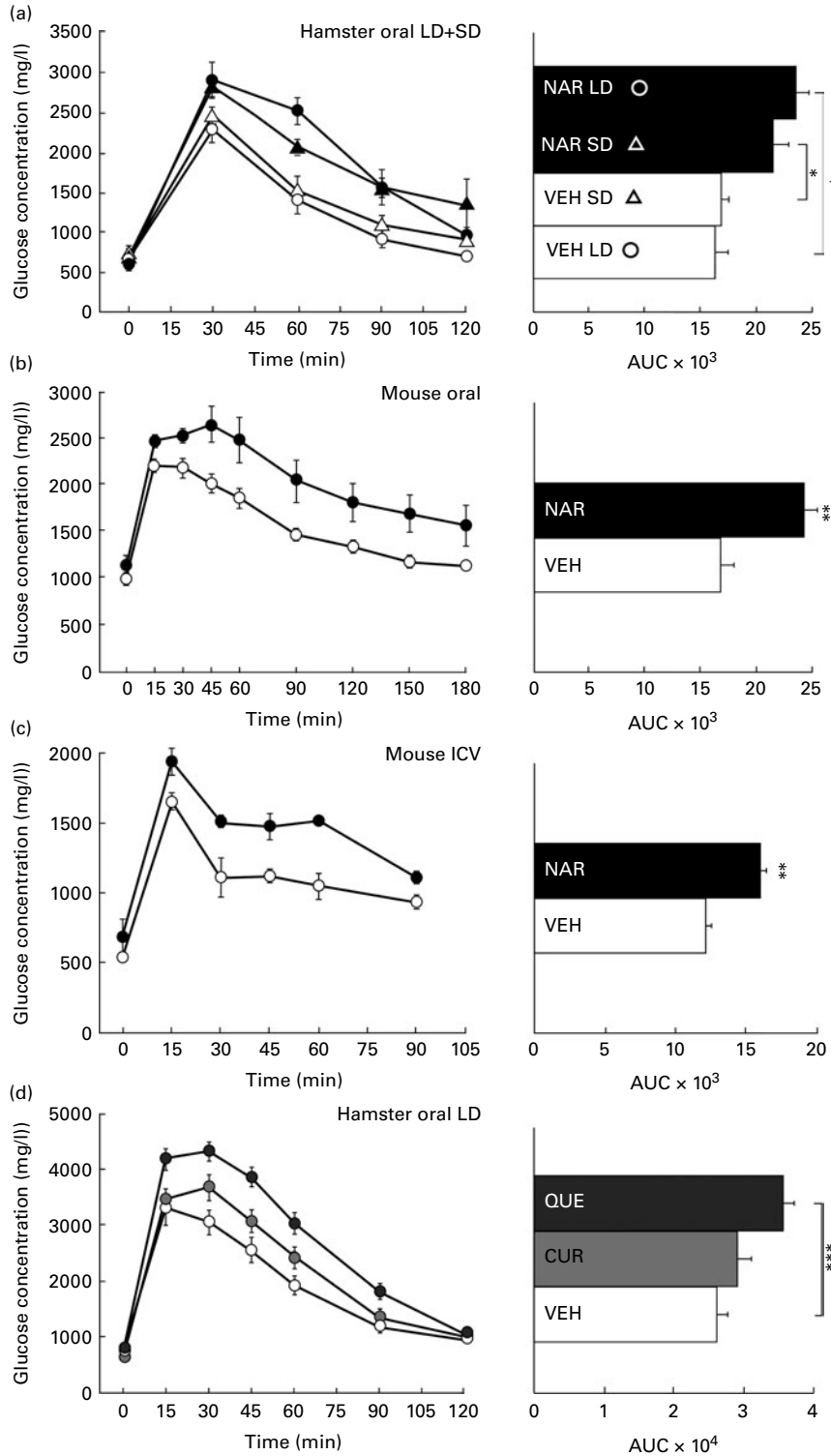


Fig. 4. Naringenin (NAR) and quercetin (QUE) significantly impaired the whole body glucose tolerance of Djungarian hamsters and mice. (a) Glucose clearance during an intra-peritoneal (ip) glucose tolerance test (ipGTT) and the corresponding area under the curve (AUC) of Djungarian hamsters treated orally with NAR (10 mg/kg body weight; long day (LD, ●) = three hamsters; short day (SD ▲) = five hamsters) or vehicle (VEH; 10% ethanol–water; LD (○) = eight hamsters; SD (Δ) = five hamsters) 15 min prior to an ip glucose injection (2 g/kg body weight). (b) Glucose clearance during an ipGTT and the corresponding AUC of 8-week-old mice (*n* 8 mice/group) pre-treated orally with NAR (10 mg/kg body weight) or VEH (10% ethanol–water) 15 min prior to an ip glucose injection (1 g/kg body weight). (c) Glucose clearance during an ipGTT and the corresponding AUC of 8-week-old mice (*n* 3 mice/group) injected centrally with either NAR (5 nmol in 5% dimethyl sulphoxide (DMSO)/artificial cerebrospinal fluid (aCSF)) or VEH (5% DMSO/aCSF) 15 min prior to an ip glucose injection (1 g/kg body weight). (d) Glucose clearance during an ipGTT and the corresponding AUC of Djungarian hamsters (LD; *n* 16–20 hamsters/group) treated orally with QUE, curcumin (CUR) (each 10 mg/kg body weight) or VEH (10% ethanol–water) 15 min prior to an ip glucose injection (2 g/kg body weight). ICV, intracerebroventricular. Values are means with their standard errors. Mean values were significantly different: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

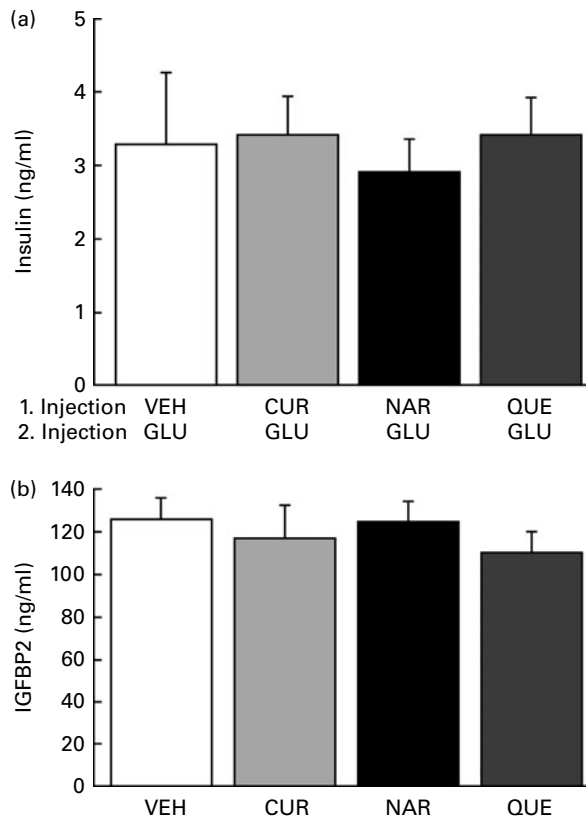


Fig. 5. Polyphenol treatment did not affect circulating levels of insulin and insulin-like growth factor-binding protein 2 (IGFBP2) in Djungarian hamsters. (a) Effect of polyphenols on glucose (GLU)-stimulated serum insulin concentrations of Djungarian hamsters (long day (LD); n 6 hamsters/group). Hamster received naringenin (NAR), quercetin (QUE), curcumin (CUR) (10 mg/kg body weight) or vehicle (VEH) (10% ethanol–water) orally, 15 min prior to an intra-peritoneal GLU injection (2 mg/kg body weight). At 30 min after GLU stimulation, blood was taken from the *vena sublingualis*. (b) Serum IGFBP2 concentration at 60 min after oral application of NAR, QUE, CUR (10 mg/kg body weight; n 6 hamsters/group) or VEH (10% ethanol–water; n 6 hamsters/group). Values are means with their standard errors.

controversial. It has been shown that naringenin and quercetin inhibited insulin signalling via the PI3K pathway *in vitro* (14,15). Additionally, both polyphenols inhibit glucose uptake in breast cancer cells (26) and in adipocytes (27,28) via blockade of GLUT4 translocation, which is regulated by PI3K (29–31). These data obtained from *in vitro* experiments strongly suggest that these two polyphenols might also impair glucose homeostasis *in vivo*. Some *in vivo* experiments, however, suggest that naringenin and quercetin exhibit anti-diabetic properties. Indeed long-term treatment with quercetin and naringenin has been shown to significantly reduce blood glucose levels in a mouse model of type 1 diabetes (9). Other studies described that both polyphenols significantly improved diet-induced insulin resistance triggered by high fructose or high fat feeding in mice and rats (8,32,33). As for naringenin and quercetin, curcumin has not been consistently described as having anti- or pro-diabetic actions. In one study, it inhibited insulin-induced GLUT 4 translocation and therefore glucose uptake in 3T3-L1 adipocytes. In another study, curcumin was described to inhibit the phosphorylation

of Akt in human prostate cancer and renal carcinoma cells (34,35). In contrast, curcumin increased the phosphorylation of Akt in melanoma cell lines (36). Moreover, some *in vivo* studies defined curcumin as an anti-diabetic agent. It improved muscle insulin resistance in a diabetic rat model (10) and prevented the increase in blood glucose during the onset of diabetes (37).

In the present study, we assessed whether these polyphenols are able to impair insulin signalling in a neuronal cell line and whole body glucose homeostasis of the Djungarian hamster and mice. Notably, no effect of photoperiod on glucose homeostasis was observed. Therefore, all experiments scrutinising the mechanism of how the flavonoids act on glucose metabolism were carried out in LD hamsters and in mice. Quercetin, which was described as a kinase inhibitor (14), consistently reduced insulin-induced activation of the PI3K pathway both *in vitro* and *in vivo*. Naringenin was equally effective *in vivo*, reducing the number of ARC pAkt immunoreactive cells after insulin stimulation to a similar level. Curcumin, however, was only able to inhibit insulin-induced activation of Akt in the neuronal cell line in a high dose. These discrepancies between *in vitro* and *in vivo* effects of the polyphenols may be explained by the substantial differences between cell culture and whole animal experiments. Circulating hormones may be required to enable naringenin to exert its full PI3K-inhibiting capacity. Also, insulin signalling in the hippocampus and in the ARC is likely to be similar as insulin and leptin receptors are expressed and PI3K is active in both regions (38–42). Leptin signalling via the PI3K pathway in the ARC and hippocampus leads to a similar behavioural response, e.g. in food intake, further substantiating the idea that signal transduction mechanisms are likely to be similar in both regions (43,44). Methodological differences in performing immunohistochemistry in the ARC and measuring pAkt by immunoblotting in hippocampal cells might also contribute to this discrepancy. A further aspect is the bioavailability and the metabolism of the polyphenols *in vivo*. While curcumin inhibited insulin-stimulated pAkt *in vitro*, but not *in vivo*, this may be due to the low bioavailability of curcumin (45,46). Naringenin, absorbed in the small intestine, is hydrolysed from naringin and only the aglycone naringenin is detectable in the circulation (47). It cannot be ruled out that secondary metabolites of naringenin, e.g. glucurono and sulfoconjugated derivatives, are bioactive *in vivo* and contribute to the inhibition of insulin signalling. However, the likelihood that these derivatives enter tissue cells is low due to their increased polarity that reduces their ability to cross the lipid cell membrane.

The ability of naringenin to impair glucose tolerance was of similar magnitude, whether the flavonoid was given ICV or orally. This together with the finding that naringenin reduced the number of pAkt immunoreactive cells after insulin stimulation suggests that the effect to impair glucose tolerance is mediated centrally. Dietary administration of naringenin to rats resulted in high detectable levels in the plasma, reaching concentrations of 35 μ M (47). It has been well described that naringenin and quercetin, both lipophilic molecules, can cross the blood–brain barrier in an *in situ* model (48–50).

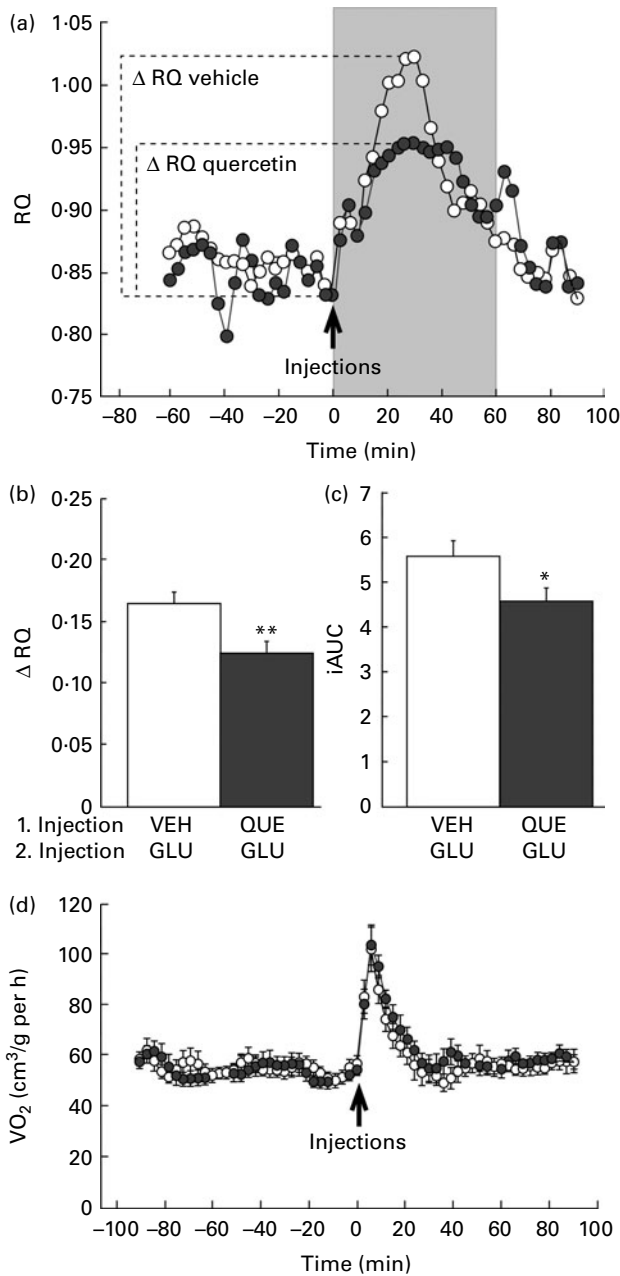


Fig. 6. Quercetin (QUE) acutely impaired glucose (GLU) utilisation in Djungarian hamsters. (a) Representative RQ of two adult female long day hamsters treated orally with either QUE (10 mg/kg body weight; ●) or vehicle (VEH; 10% ethanol-water; ○) 15 min prior to an intra-peritoneal (ip) GLU injection (2 mg/kg body weight). Injection phase is marked with an arrow, time period for the incremental area under the curve (iAUC) calculation (shown in Fig. 5(b)) is highlighted in light grey and calculation of ΔRQ (shown in Fig. 5(c)) is represented by dotted lines. (b) Maximal increase of the RQ (ΔRQ) of these hamsters (n 10 hamsters/group) after QUE or VEH pre-treatment. (c) iAUC of hamsters (n 10 hamsters/group) treated orally with QUE (10 mg/kg body weight) or VEH (10% ethanol-water) 15 min prior to an ip GLU injection (2 mg/kg body weight). (d) Oxygen consumption of Djungarian hamsters (n 10 hamsters/group) treated orally with QUE (10 mg/kg body weight; ●) or VEH (10% ethanol-water; ○) 15 min prior to an ip GLU injection (2 mg/kg body weight). Injection phase is marked with an arrow. Values are means with their standard errors. Mean values were significantly different: * $P < 0.05$, ** $P < 0.01$.

Furthermore, it has been shown that, specifically in the brain, a high retention of the aglycone naringenin and not its metabolites occurred even as long as 18 h after a naringenin-enriched meal⁽⁵¹⁾. Also, dietary naringenin inhibits the proliferation of cerebrally implanted glioma cells in rats⁽⁵²⁾, suggesting that the molecule is bioactive in the brain tissue.

Quercetin was also able to impair glucose tolerance in hamsters. Furthermore, it reduced glucose utilisation measured by a reduction in the RQ. Quercetin and naringenin have a similar chemical structure (flavan backbone (2-phenylchroman)) and both inhibit PI3K *in vitro*^(14,15) as well as cytochrome P450 *in vivo* ((CYP1A2)⁽⁵³⁾). The functional group of quercetin (chromone moiety) directly blocks the ATP-binding site of PI3K⁽¹⁴⁾ and is thus able to reduce enzyme activity. The same inhibitory functional group can be found in naringenin and might also be able to reduce PI3K activity. These parameters strongly suggest that both naringenin and quercetin have a similar mode of action. Thus, it is plausible that quercetin impairs glucose tolerance and glucose utilisation via inhibition of hypothalamic insulin signalling. A reduction in insulin-induced pAkt in the ARC after quercetin treatment supports this idea. An acute central inhibition of insulin signalling by the flavonoids may be largely responsible for the observed effects on glucose metabolism as ICV naringenin is as effective as oral administration. However, we cannot exclude the fact that inhibition of PI3K activity in the periphery also contributes to impairment in whole body glucose metabolism. It has been described that flavonoids inhibit glucose uptake in adipocytes^(15,27) and glucose transport by human intestinal cells⁽⁵⁴⁾. Controversially, however, naringenin was described to increase glucose uptake in muscle cells via a mechanism involving 5'AMP-activated protein kinase⁽⁵⁵⁾.

In addition to affecting glucose metabolism, oral naringenin and quercetin treatment led to a body weight gain and an increase in food intake. It is known that PI3K is involved in the regulation of energy homeostasis. Activation of this pathway reduced body weight in mice⁽⁵⁶⁾. Absence of the catalytic subunit p110 α or β of PI3K in pro-opiomelanocortin neurons led to an increase in food intake and body weight^(57,58). Quercetin reduces PI3K enzyme activity via inhibition of the ATP-binding site⁽¹⁴⁾, which is located on the catalytic subunit p110⁽⁵⁹⁾. The specific inhibition of this binding site by naringenin and quercetin might lead to the observed anabolic effect. However, the impairment in glucose metabolism after quercetin or naringenin treatment cannot be attributed to this observed anabolic effect as the intra-peritoneal glucose tolerance test was performed immediately (15 min) after flavonoid application. We cannot exclude that chronic treatment with quercetin or naringenin might influence peripheral insulin sensitivity based on a possible chronic increase in body weight. However, recently we could show in *Lep^{ob/ob}* mice that apparently not the body weight increase *per se* leads to impaired glucose homeostasis, rather the absence of central leptin action appears to be the contributing factor to the development of glucose intolerance⁽²¹⁾.

The precise nature of how the polyphenols act on energy and glucose metabolism remains incompletely understood. Here, we could show that insulin- and basal IGF1P2

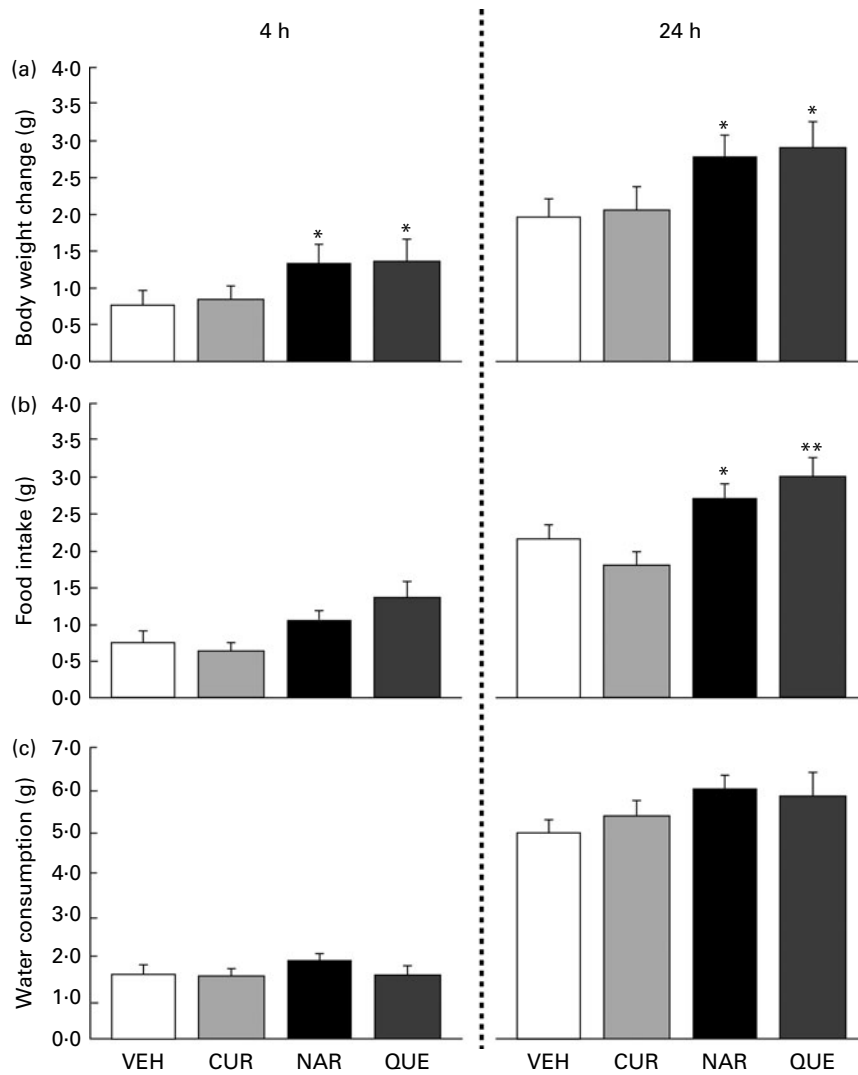


Fig. 7. Naringenin (NAR) and quercetin (QUE) significantly increased body weight gain and food intake of long day hamsters. (a) Body weight gain, (b) food intake and (c) water consumption 4 and 24 h after oral application of NAR, QUE, curcumin (CUR; 10 mg/kg; *n* 9 hamsters/group) or vehicle (VEH) (10% ethanol–water; *n* 8 hamsters). Values are means with their standard errors. Mean values were significantly different: **P* < 0.05, ***P* < 0.01.

concentrations did not change after polyphenol treatment. We measured IGFBP2 due to accumulating evidence showing that the growth hormone/insulin-like growth factor axis is involved in the regulation of glucose homeostasis. It has been recently shown that the central effect of leptin to ameliorate glucose homeostasis in leptin-deficient diabetic mice might be mediated via leptin-triggered release of IGFBP2 by the liver⁽²⁴⁾. The present data suggest that it seems unlikely that the polyphenols also act on glucose metabolism via affecting IGFBP2 release. We cannot exclude, however, that basal IGFBP2 levels in blood might change after chronic polyphenol treatment.

The administered dietary dose of, e.g. naringenin, in the present experiments was equal to approximately 1 ml grapefruit juice. Extrapolated to human dietary intake of naringenin, this treatment would approximately equal one glass of grapefruit juice/d^(60,61). The average daily flavonoid intake in the USA was reported to be approximately 1 g/person⁽⁶²⁾.

This suggests that naringenin and quercetin are highly potent inhibitors of PI3K *in vivo*. Acute dietary consumption of grapefruits and green tea might lead to a similar plasma load, as observed in animals, and consequently might impair glucose metabolism. Whether chronic consumption of these nutrients might contribute to the development of type 2 diabetes remains a matter of debate. Future experiments involving chronic polyphenol treatment and other animal models that exhibit a profound phenotype of insulin resistance are required to solve this important issue.

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the data and wrote the manuscript. K. L. performed metabolic measurements with the expertise of G. H. D. S. and J. S. assisted by performing and analysing the immunohistochemistry. G. K. G. and S. S. performed *in vitro* experiments. G. K. G. was also involved in writing sections of the manuscript. N. H. reviewed/edited the manuscript. A. T. designed the study and reviewed/edited the manuscript. All authors approved the final version to be published and have no conflict of interest to declare.

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