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ORIGINAL COMMUNICATION

Effects of changes in hydration on protein, glucose and lipid metabolism in man: impact on health

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Alterations of cell volume induced by changes of extracellular osmolality have been reported to regulate intracellular metabolic pathways. Hypo-osmotic cell swelling counteracts proteolysis and glycogen breakdown in the liver, whereas hyperosmotic cell shrinkage promotes protein breakdown, glycolysis and glycogenolysis. To investigate the effect of acute changes of extracellular osmolality on whole-body protein, glucose and lipid metabolism *in vivo*, we studied 10 male subjects during three conditions: (i) hyperosmolality was induced by fluid restriction and intravenous infusion of hypertonic NaCl (2–5%, wt/vol) during 17 h; (ii) hypo-osmolality was produced by intravenous administration of desmopressin, liberal water drinking and infusion of hypotonic saline (0.4%); and (iii) the iso-osmolality study comprised oral water intake *ad libitum*. Plasma osmolality increased from 285 ± 1 to 296 ± 1 mosm/kg ($P < 0.001$ during hyperosmolality, and decreased from 286 ± 1 to 265 ± 1 mosm/kg during hypo-osmolality ($P < 0.001$). Total body leucine flux ([1-^{13}C]leucine infusion technique), reflecting whole-body protein breakdown, as well as whole-body leucine oxidation rate (irreversible loss of amino acids) decreased significantly during hypo-osmolality. The glucose metabolic clearance rate during hyperinsulinaemic–euglycemic clamping increased significantly less during hypo-osmolality than iso-osmolality, indicating diminished peripheral insulin sensitivity. Glycerol turnover (2-[^{13}C]glycerol infusion technique), reflecting whole-body lipolysis, increased significantly during hypo-osmolar conditions. The results demonstrate that the metabolic adaptation to acute hypo-osmolality resembles that of acute fasting, that is, it results in protein sparing associated with increased lipolysis, ketogenesis and lipid oxidation and impaired insulin sensitivity of glucose metabolism.
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Introduction

Hepatic cell metabolism is regulated by substrates, enzymes, hormones and nerves. Another recently described mechanism regulates cellular metabolism via changes of cell volume (the 'cell volume hypothesis'). Liver cell volume is influenced by changes of extracellular osmolality, substrate uptake into hepatocytes, hormones and oxidative stress (Häussinger *et al*, 1993, 1994; Häussinger, 1996). Factors leading to cell swelling are for example, extracellular hypo-osmolality, elevated plasma insulin, glutamine, glycine and alanine, whereas factors inducing cell shrinkage are hyperosmolal plasma, increased plasma glucagon concentrations, vasopressin and serotonin. Exposure of hepatocytes to a hypo-osmotic environment leads initially to cell swelling, but then within minutes cells display a regulatory volume decrease due to opening of K^+, Cl^- and HCO_3^- channels. In contrast, cell shrinkage following exposure to hyperosmotic fluids results in a regulatory volume increase due to activation of Na^+–H^- antiport activity, Na^+–K^+–ATPase and Cl^-–HCO_3^- exchanges (Graf *et al*, 1996). Both these regulatory mechanisms are completed within minutes, but are not able to restore the initial cell volume. Thus, cells remain in a slightly swollen or shrunken state. The extent of these cell volume changes modifies cellular function. As expected from this model, it has been shown *in vitro* that cell swelling and shrinkage lead to opposite metabolic effects on protein, carbohydrate and lipid metabolism.

Cell shrinking induced by hyperosmolality promotes proteolysis in liver cells, whereas hypo-osmotic cell swelling counteracts protein catabolism (Häussinger *et al*, 1990). Hypo-osmotic cell swelling can mimic amino-acid-induced

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stimulation of glycogen synthesis (Baquet *et al*, 1990; Häussinger *et al*, 1990). Changes in cell size appear to serve as a mediator of insulin-, glucagon- and catecholamine-regulated lipolysis (Häussinger, 1996).

These *in vitro* results are in agreement with clinical observations. Dehydration in decompensated diabetes mellitus—a model of hyperosmolality—is associated with protein catabolism and insulin resistance of glucose metabolism (Hellerstein, 1995). Further, critically ill patients demonstrated progressive cellular dehydration associated with proteolysis (Finn *et al*, 1996).

However, clinical investigations in physiological states, in which cell swelling and shrinkage may be expected are rare, and metabolic effects due to acute changes of extracellular osmolality have not been assessed previously in humans.

Based on these data, we examined protein, glucose and lipid kinetics in the hyper-, iso- and hypo-osmolal states in healthy men (Berneis *et al*, 1999; Bilz *et al*, 1999).

We expected a diminished protein catabolic rate during hypo-osmolal conditions, and increased proteolysis during hyperosmolality, respectively.

We hypothesised according to the *in vitro* data that hypo-osmolal cell swelling would lead to diminished glucose turnover in analogy to insulin-induced cell swelling.

Adipose tissue lipolysis induced by hormone-sensitive lipase is mainly regulated by insulin, glucagon and catecholamines (Arner, 1996). Therefore, the present studies should also examine whether whole-body lipolysis and lipid oxidation are affected by hypo-osmolar conditions (Bilz *et al*, 1999).

Methods

In all, 10 healthy young male volunteers were observed during conditions of iso-osmolality, hyperosmolality and hypo-osmolality in randomised order (study protocol: Figure 1). Baseline kinetics of leucine, glucose and glycerol were measured from 17:00 to 20:00. Hypo-osmolality, hyperosmolality and iso-osmolality studies lasted from 20:00 to 13:00. At 20:00 the subjects were served a standard meal (600 kcal) and remained fasting thereafter. In the hypo-osmolality study, the subjects received 4 μg desmopressin intravenously at 20:00 and at 08:00 the next day, and in addition they drank 2.4 l/12 h of tap water. At 08:00 in the morning an infusion of hypotonic saline (0.4%, 200 ml/h from 08:00 to 13:00) was started. Hyperosmolality was induced by infusion of hypertonic saline (2% NaCl (wt/vol) 1 ml/kg/h from 20:00 to 08:00 and 5% NaCl 200 ml/h from 08:00 to 13:00). Iso-osmolality was maintained by access to oral water *ad libitum* during the entire study. Plasma concentrations of sodium and osmolality were measured repeatedly during all studies.

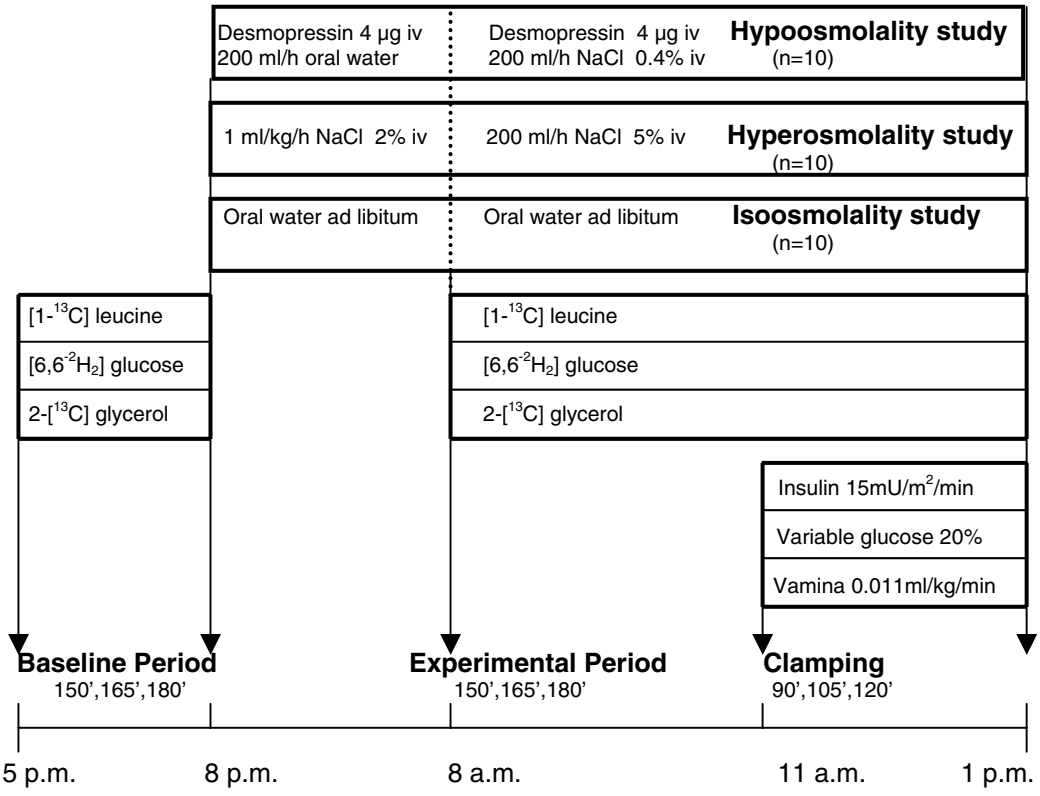

Figure 1 Experimental protocol of the studies. Vamina=standard mixed amino-acid solution (7% wt/vol).

Whole-body protein kinetics were assessed using the [1-^{13}C]leucine infusion technique and the reciprocal pool model, assuming steady-state conditions during the baseline period, the experimental period and during a hyperinsulinaemic glucose-clamping period. The essential amino acid leucine is released from endogenous proteins, and leucine oxidation is used as a parameter of irreversible protein catabolism. The effects of hyper- and hypo-osmolality on [1-^{13}C] enrichment of α-ketoisocaproate and of CO_2 during [1-^{13}C]leucine tracer infusion were measured. Total leucine flux was calculated by dividing the infusion rate of [1-^{13}C]leucine by the plasma α-[1-^{13}C]ketoisocaproate (α-KIC) tracer-to-tracee ratio according to the reciprocal pool model (Cobelli *et al*, 1987; Horber *et al*, 1989). Leucine oxidation rate was calculated by dividing the product of $^{13}CO_2$ atom percent excess and CO_2 production rate in expired air by plasma α-[1-^{13}C]ketoisocaproate tracer-to-tracee ratio.

Glucose kinetics (glucose metabolic clearance rate (MCR) and endogenous glucose rate of appearance (glucose R_a) representing mainly hepatic glucose production) were determined using [6,6-$^2H_2$]glucose infusions during the baseline period, the experimental period and the hyperinsulinaemic glucose-clamping period. Endogenous glucose R_a was calculated by dividing the [6,6-$^2H_2$]glucose infusion rate by the plasma glucose tracer-to-tracee ratio. During glucose clamping, total plasma glucose R_a was the sum of the glucose infusion rate and endogenous glucose R_a. Glucose metabolic clearance rate was calculated by glucose R_a divided by the corresponding plasma glucose concentration.

Lipolysis was assessed by measuring glycerol rate of appearance (glycerol R_a) by using a stable isotope method (2-[^{13}C]glycerol infusions) and indirect calorimetry. Glycerol kinetic studies were performed during the baseline period at the beginning of the first experimental day and during the experimental period and the hyperinsulinaemic glucose-clamp period after an overnight fast on the second experimental day. Since stable levels of plasma glycerol concentrations and enrichment were present, glycerol R_a was calculated by dividing the infusion rate of 2-[^{13}C]glycerol with the plasma glycerol tracer-to-tracee ratio (Beylot *et al*, 1987). In addition, plasma concentrations of FFA, acetoacetate, β-hydroxybutyrate, insulin, C-peptide, glucagon, epinephrine and norepinephrine were measured. Carbohydrate and fat oxidation, nonprotein respiratory quotients (calculated by dividing CO_2 production by O_2 consumption) and energy expenditure were determined using indirect calorimetry and standard formulas assuming a nitrogen excretion rate of 13 g/day (Jéquier & Felber, 1987).

Results

Serum sodium, plasma osmolality and fluid balance

During iso-osmolality plasma sodium and osmolality remained unchanged. In the hyperosmolal state plasma sodium increased from 142 ± 0.2 to 149 ± 0.4 mmol/l ($P<0.0001$), and osmolality increased from 283.4 ± 0.5 to 296.4 ± 0.7 mosm/kg ($P<0.0001$), whereas during hypo-osmolality plasma sodium and osmolality decreased significantly from 142 ± 0.4 to 131 ± 0.5 mosm/kg, and from 286 ± 1 to 265 ± 1 mosm/kg ($P<0.0001$), respectively (Figure 2). Body weight (kg) remained unchanged during iso-osmolar (69.5 ± 4.8 and 68.9 ± 4.6) and hyperosmolar conditions (69.4 ± 4.6 and 69.2 ± 4.5), but increased in the hypo-osmolality group from 69.1 ± 4.8 to 70.7 ± 4.8 ($P<0.005$). Fluid balance as assessed by fluid administration and urinary output was positive in the hypo-osmolar group ($+1.56 \pm 0.17$ l) and nearly unchanged during hyperosmolality.

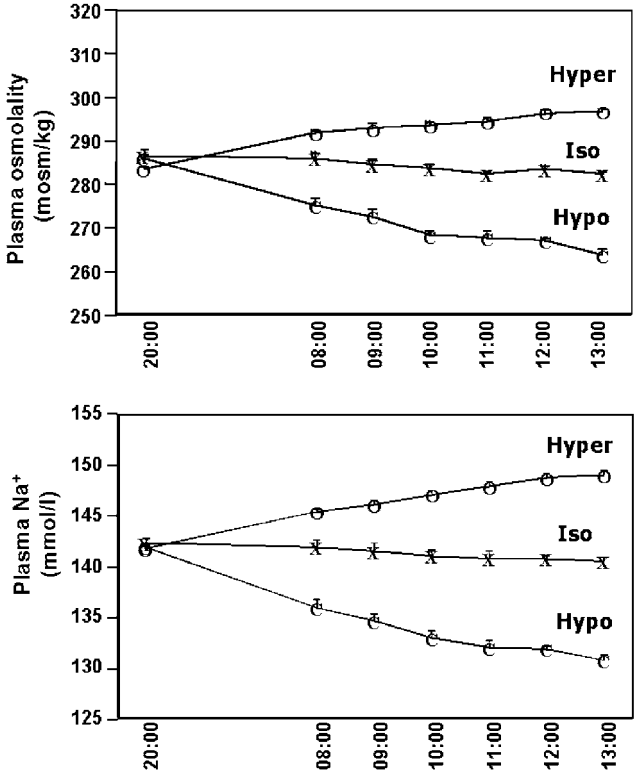

Figure 2 Plasma osmolality (mosm/kg) and sodium plasma concentrations (mmol/l) during Hyper: hyperosmolality, Iso: iso-osmolality, Hypo: hypo-osmolality. Data are means $\pm$ SEM; n=10 (Berneis *et al*, 1999).

Leucine kinetics

Leucine oxidation (Figure 3, upper panel) decreased during the hypo-osmolality study from baseline to the experimental period from 0.34 ± 0.03 to 0.27 ± 0.01 μmol/kg/min ($P<0.03$ *vs* baseline, $P<0.005$ *vs* iso-osmolality) and remained unchanged during the other two studies. It was significantly lower during hypo-osmolality than during the iso- and hyperosmolality studies ($P<0.005$). Leucine flux (Figure 3(b), bottom panel) decreased during hypo-osmolality from 1.9 ± 0.05 μmol/kg/min at baseline to 1.79 ± 0.06 μmol/kg/

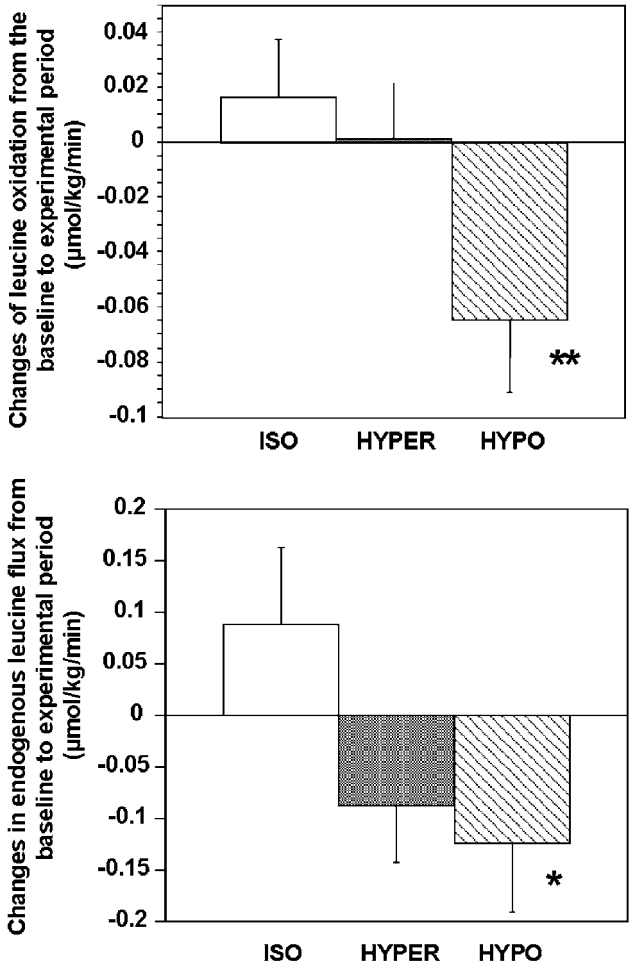

Figure 3 Changes of leucine oxidation rate (μmol/kg/min) and of endogenous leucine flux (μmol/kg/min) from the baseline to the experimental periods. Iso: iso-osmolality; Hyper: hyperosmolality; Hypo: hypo-osmolality $^{*}P<0.05$; $^{**}P<0.005$ *vs* Iso. Data are means±SEM; $n=10$ (Berneis *et al*, 1999).

min at the end of the experimental period ($P<0.02$ *vs* iso-osmolality), indicating reduced reversible protein breakdown. It remained unchanged during the course of the iso-osmolality and hyperosmolality studies. Whole-body protein synthesis (represented by nonoxidative leucine disappearance, and calculated by subtracting the rate of leucine oxidation from total leucine flux) during hypo-osmolality was not altered. Thus, the net protein balance improved during the hypo-osmolal state.

Glucose kinetics

Plasma glucose concentrations differed during the experimental periods significantly between the iso-, hyper- and hypo-osmolal states (Table 1). Glucose concentrations were higher during hyperosmolality (5.1 mmol/l) and lower during hypo-osmolality (4.7 mmol/l) compared to iso-osmolality (4.9 mmol/l) (hyper- and hypo-osmolality *vs* iso-osmolality: $P<0.03$).

Table 1 Glucose plasma concentrations and kinetics (Berneis et al, 1999)

Parameter	*Baseline*	*Experimental*	*Clamp*
Iso-osmolality study			
Glucose plasma concentrations (mmol/l)	4.8±0.2	4.9±0.1	4.9±0.1
Endogenous glucose R_a (μmol/kg/min)	13±0.7	11.2±0.4[c]	5.3±0.7
Glucose MCR (ml/kg/min)	2.8±0.1	2.3±0.l[c]	4.4±0.4[d]
Hyperosmolality study			
Glucose plasma concentrations (mmol/l)	4.9±0.05	5.1±0.05[a]	4.9±0.1
Endogenous glucose R_a (μmol/kg/min)	13.5±0.6	12.4±0.4[a]	6±0.6
Glucose MCR (ml/kg/min)	2.8±0.1	2.5±0.l[c]	4.2±0.4[d]
Hypo-osmolality study			
Glucose plasma concentrations (mmol/l)	4.8±0.1	4.7±0.05[a]	4.8±0.1
Endogenous glucose R_a (μmol/kg/min)	12.8±0.1	11.1±0.3	4.6±0.6
Glucose MCR (ml/kg/min)	2.7±0.2	2.4±0.1	3.3± 0.2[b,d]

Data are means±SE; $n=10$ subjects/group. R_a, rate of appearance; MCR, metabolic clearance rate.
[a]$P<0.05$ *vs* iso-osmo (paired *t*-tests).
[b]$P<0.05$ *vs* iso-osmolality (repeated-measure ANOVA).
[c]$P<0.05$ *vs* baseline (paired *t*-tests).
[d]$P<0.005$ *vs* experimental (paired *t*-tests).

Endogenous glucose R_a remained unchanged during hypo- and hyperosmolality, in contrast to iso-osmolality where it decreased significantly ($P<0.02$). Endogenous glucose R_a during the experimental period was higher in the hyperosmolal state than during iso- and hypo-osmolality ($P<0.05$). Glucose MCR decreased from the baseline to the experimental periods during iso- and hyperosmolality, but not during hypo-osmolality. During euglycaemic clamping glucose MCR increased in all studies, but was highest in the iso-osmolal state (4.4 ml/kg/min) and lowest in the hypo-osmolal state (3.3 ml/kg/min). The mean glucose infusion rate needed to maintain euglycaemia was 14.9 μmol/kg/min in the iso-osmolality study, 14.4 μmol/kg/min during hyperosmolality, but only 10.5 μmol/kg/min during hypo-osmolality ($P<0.01$ *vs* iso-osmolality). Plasma insulin concentrations were lower during the experimental period of hypo-osmolality than during iso- and hyperosmolality ($P<0.05$).

Glycerol kinetics, plasma free fatty acids and ketone bodies

In the baseline study, glycerol R_a was similar during iso-osmolality and hypo-osmolality. During the experimental

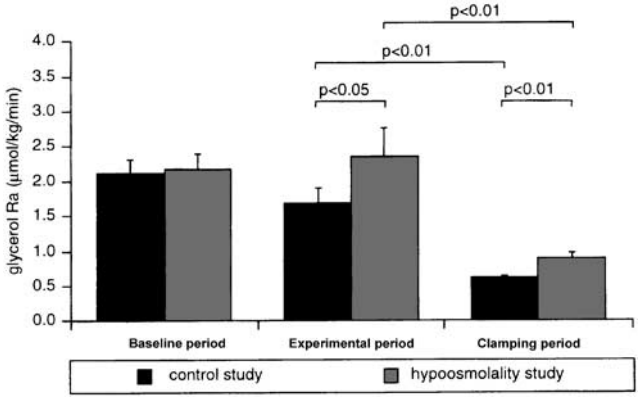

Figure 4 Glycerol rate of appearance (R_a; μmol/kg/min) during the baseline period, the experimental period and the clamping period (euglycaemic clamping during hyperinsulinaemia). Control study=iso-osmolality. Results are means±SEM; *n*=7 (Bilz *et al*, 1999).

period after a 14 h fast the glycerol R_a was higher during hypo-osmolality than during iso-osmolality (2.35±0.40 *vs* 1.68±0.21 μmol/kg/min, P=0.03). This difference was also apparent during the clamp period (0.90±0.08 *vs* 0.61±0.03 μmol/kg/min, P=0.002). Compared with the experimental period, the glycerol R_a was lower during the glucose-clamp period of both the iso- and the hypo-osmolality studies ($P<0.01$; Figure 4).

There were no significant differences of plasma glycerol, FFA, acetoacetate, β-hydroxybutyrate, insulin, C-peptide and glucagon plasma concentrations between the hypo-osmolal and the control states. Plasma epinephrine concentrations did not differ between the experimental and the clamp periods, when hypo-osmolality and the control studies were compared. Plasma insulin and glucagon increased in the iso- and hypo-osmolality studies during the glucose-clamping period compared to the experimental period in a similar extent without statistically significant differences. Plasma norepinephrine concentrations were lower during hypo-osmolality, both during the baseline and the clamp period ($P<0.05$), but showed no changes within both protocols.

Discussion

The present studies examined for the first time the effect of acute hyper- and hypo-osmolality on whole-body protein, glucose and lipid metabolism in humans. The changes of extracellular osmolality induced presumably a modest state of cell swelling or shrinking, respectively (Graf & Häussinger, 1996).

Hypo-osmolality compared to iso-osmolality led to diminished leucine release from endogenous proteins (representing protein breakdown), and decreased leucine oxidation (indicating irreversible catabolism). The net protein synthesis (nonoxidative leucine disappearance) during hypo-osmolality was not altered, resulting in a net positive protein balance. The protein sparing effects of hypo-osmolality are in accordance with *in vitro* data showing increased protein synthesis and amino-acid uptake and decreased proteolysis (Häussinger & Lang, 1991; Stoll *et al*, 1992; Häussinger *et al*, 1994). In contrast, the studies did not reveal an increase in leucine flux and oxidation during hyperosmolality, as suggested by the *in vitro* experiments. This may be due to the short experimental period to detect changes of protein breakdown, or due to the fact that the increase in osmolality was insufficient to induce a significant protein catabolism.

Plasma glucose concentrations and endogenous glucose R_a (representing mainly hepatic glucose production) were increased during hyperosmolality compared to iso-osmolal conditions, while hypo-osmolality induced a decrease of plasma glucose concentrations. Further, euglycaemic clamping during a hypo-osmolal state resulted in a diminished increase of glucose MCR, indicating diminished insulin sensitivity of peripheral glucose metabolism. The present experiments indicate a glucose sparing effect during hypo-osmolality, and an increase in hepatic glucose production, with increased plasma glucose levels during hyperosmolality. As insulin leads to cell swelling and glucagon to cell shrinkage, their metabolic effects may be explained in part by their effects on cell volume. The present data are in accordance with *in vitro* experiments, suggesting that glycogenolysis was reduced as a consequence of hypo-osmolal; liver cell swelling (Häussinger & Lang, 1991; Stoll *et al*, 1992; Häussinger *et al*, 1994); they support the cell volume hypothesis in an *in vivo* model.

Further, to investigate carbohydrate *vs* fat utilisation, indirect calorimetry was performed. Carbohydrate oxidation was lower and utilisation of fat higher during hypo-osmolality, in contrast to iso- and hyperosmolality. These data support a glucose-sparing effect of the hypo-osmolal state.

On the other hand, the finding of increased lipolysis was in accordance with the observed increase in plasma non-esterified fatty acid concentrations, suggesting a lipolytic effect of hypo-osmolality on triglycerides in adipose tissue.

The plasma concentrations of lipolysis regulating hormones (insulin, epinephrine) were not different between the protocols, indicating that the lipolytic effect of hypo-osmolality was independent of these primary regulators. These observations are in accordance with *in vitro* data, suggesting that changes of cell size serve as a mediator of insulin-, glucagon-and catecholamine-regulated lipolysis (Häussinger, 1996). The finding of a significantly higher glycerol R_a during hyperinsulinaemic euglycaemic clamping strongly suggests that the antilipolytic action of insulin was partially overcome by hypo-osmolar conditions *in vivo*. This, however, is in contrast to previous *in vitro* findings demonstrating that insulin and hypo-osmolality exert similar metabolic effects. All previous studies on the effects of hyper- and hypo-osmolality on cell metabolism have been performed using perfused livers or isolated hepatocytes. Although lipolysis occurs in all triglyceride-storing tissues, adipose tissue lipolysis reflects the majority of lipolytic

activity *in vivo*, and this compartment has not been investigated previously *in vitro*.

In conclusion, hypo-osmolality promotes lipolysis combined with counteracting proteolysis and glycogenolysis, whereas hyperosmolality induces glycogenolysis. In terms of human fuel regulation, this indicates that the effects of acute hypo-osmolality resemble those of acute fasting. The results could be interpreted in the way that high fluid intake leading to hypo-osmolality induces a reduction of body fat stores. In contrast to *in vitro* findings, we could not find a proteolytic effect of hyperosmolality *in vivo*. This indicates that metabolic principles derived from studies performed *in vitro* have to be confirmed *in vivo* before any conclusions can be drawn regarding their significance in humans.

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