Effects of hyper- and hypoosmolality on whole body protein and glucose kinetics in humans

KASPAR BERNEIS,1 RONALD NINNIS,1 DIETER HÄUSSINGER,2 AND ULRICH KELLER1
1Departments of Research and of Internal Medicine, University Hospital Basel, 4031 Basel, Switzerland; and 2Department of Internal Medicine, University Hospital Düsseldorf, 40225 Düsseldorf, Germany

Berneis, Kaspar, Ronald Ninnis, Dieter Häussinger, and Ulrich Keller. Effects of hyper- and hypoosmolality on whole body protein and glucose kinetics in humans. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E188–E195, 1999.—To investigate the effect of acute changes of extracellular osmolality on whole body protein and glucose metabolism, we studied 10 male subjects during three conditions: hyperosmolality was induced by fluid restriction and intravenous infusion of hypertonic NaCl [2–5% (wt/vol)] during 17 h; hypoosmolality was produced by intravenous administration of desmopressin, liberal water drinking, and infusion of hypotonic saline (0.4%); and the isoosmolality study consisted of ad libitum oral water intake by the subjects. Leucine flux ([1-13C]leucine infusion technique), a parameter of whole ad libitum oral water intake by the subjects. Leucine flux was produced by intravenous administration of hypertonic NaCl [2–5%; (wt/vol)] during 17 h; hypoosmolality results in protein sparing associated with increased lipolysis and lipid oxidation and impaired insulin sensitivity.

METHODS

Subjects. Written informed consent was obtained from 10 healthy young male volunteers aged 25 ± 1 yr with a body mass index of 23.0 ± 0.8 kg/m². Their medical history, physical examination, and routine laboratory tests before the studies provided no evidence of cardiopulmonary, renal, hepatic, or metabolic diseases. The subjects were on no medication and did not perform vigorous exercise during the study period. The study protocol was reviewed and approved by the ethical committee of the Basel University Hospital.

Procedures. Each subject underwent three sets of studies (hyperosmolality, hypoosmolality, and isoosmolality) in randomized order and with intervals of ≥1 wk in between (Fig. 1). Each subject remained fasting after 11 AM and was admitted at 4:30 PM to the metabolic study unit of the hospital. Thereafter, baseline kinetics were measured by placing a Teflon cannula into the right antecubital vein for blood sampling. The hand was kept in a thermostat-controlled warming chamber at −55°C, allowing arterialization of hand venous blood (35). Blood and breath samples were subsequently obtained to determine background isotopic enrichments of plasma [2H2]glucose, [1-13C]leucine, α-[1-13C]ketoglutarate, and breath 13CO2, followed by primed-continuous infusions of [1-13C]leucine (3 µmol/kg bolus; 0.06 µmol·kg−1·min−1 infusion; 99% enriched; sterile and pyrogen free; Mass Trace, Somerville, MA) and of [6,6-2H2]glucose (3 mg/kg bolus; 0.04 mg·kg−1·min−1 infusion;
98% enriched; sterile and pyrogen free; Mass Trace) administered during 3 h. A single injection of sodium [1-13C]bicarbonate (1.76 µmol/kg; 99% enriched; sterile and pyrogen free; Mass Trace) was used to accelerate 13C labeling of the bicarbonate pool. After 150 min of tracer equilibration, blood and breath samples were obtained in 15-min intervals during 30 min (baseline kinetics). At 8 PM, the subjects were served a standard meal (600 kcal) and remained fasting thereafter until the end of the study on the following day.

In the hyperosmolality study, the subjects were instructed not to drink after 8 PM after the first day of the study; during the night, they received 1 ml·kg\(^{-2}\)·h\(^{-1}\) of a saline [2% NaCl (wt/vol)] infusion, and at 8 AM, they received an infusion of 200 ml/h of 5% NaCl. In the hypoosmolality study, the subjects received 4 µg desmopressin (Minirin) intravenously at 8 PM on the first study day and at 8 AM on the second study day, respectively, and they were instructed to drink 2–2.5 liters of tap water during the night. A 0.4% NaCl (200 ml/h) infusion was started in the morning until the end of the study. The isoosmolality study consisted of an identical protocol in which osmolality was maintained constant throughout the study by access to oral water ad libitum. In all studies, plasma Na\(^+\) concentrations and osmolality were measured before the treatment was started and hourly after 8 AM on the second day. The subjects were supervised throughout the study by a physician.

Leucine and glucose kinetics were measured a second time on study day 2 in an identical fashion, starting at 8 AM and continuing during 5 h. After the total experimental period of 180 min, insulin (Actrapid, Norvonomdics, Küssnacht) was infused continuously at 60 mU·m\(^{-1}\)·h\(^{-1}\) during 3 min, and then at 15 mU·m\(^{-1}\)·min\(^{-1}\) during 117 min (7). In addition, 20% glucose (wt/vol) was infused at variable rates and adjusted every 5–10 min according to rapidly measured plasma glucose concentrations to maintain euglycemia; a standard mixed amino acid solution (Vamina 10%, Pharma- cia, Stockholm, Sweden) was administered intravenously at a rate of 0.0144 ml·kg\(^{-1}\)·min\(^{-1}\), corresponding to a leucine infusion rate of 0.65 µmol·kg\(^{-1}\)·min\(^{-1}\). The composition of Vamina (in g/l) was 2.5 aspartate, 4.2 glutamate, 5.9 glycin, 12.0 alanine, 8.4 arginine, 0.42 cysteine, 5.1 histidine, 4.2 isoleucine, 5.9 leucine, 6.8 lysine, 4.2 methionine, 5.9 phenylalanine, 5.1 proline, 3.2 serine, 4.2 threonine, 1.4 tryptophane, 0.17 tyrosine, and 5.5 valine. The amino acid solution was enriched with 0.295 g [1-13C]leucine per 1,000 ml solution to 5% tracer-to-tracee ratio (TTR) to maintain plasma a-KIC TTR during infusion unchanged. Plasma was rapidly obtained by refrigerated centrifugation (4°C) and was stored at 70°C until later assay. Expired air was collected into gastight 20-ml glass tubes (Vacutainer, Becton-Dickinson, Meylan, France) for later 13CO\(_2\) analysis.

Analytical methods. All tracer infusates were ultrafiltrated (0.1 µm) and analyzed by gas chromatography-mass spectrometry (GC-MS model 5890/5790, Hewlett-Packard, Palo Alto, CA) for tracer concentration, isotopic enrichment, and chemical purity. Plasma TTR of [1-13C]leucine and of a-[1-13C]KIC was measured by GC-MS selected ion monitoring (39). Plasma concentrations of leucine and a-KIC were determined by the same methodology with [2H\(_7\)]leucine and [2H\(_3\)]a-KIC as internal standards, respectively. Isotopic enrichment of 12CO\(_2\) in expired air was measured by isotope ratio mass spectrometry (SIRA series II, VG Isotech, Cheshire, UK). CO\(_2\) production rate (V\(_{\text{CO}_2}\)) was determined by indirect calorimetry with a ventilated hood metabolic monitor (Deltatrac II MBM-200, Datex, Helsinki, Finland). Plasma arginine vasopressin was measured by RIA as described by Liard et al. (32). Plasma glucose concentrations were measured with glucose oxidase and a hydrogen peroxide sensor (glucose analyzer 2300 STAT Plus, YSI, Yellow Springs, OH). Plasma sodium and potassium concentrations were measured by indirect potentiometry (Du Pont Dimension AR, Dade, Düdingen, Switzerland), and plasma osmolality was measured by cryoscopic technique (micro osmometer 3 MO, Advanced Instruments, Norwood, MA).

Plasma concentrations of C-peptide (23), glucagon, and insulin were measured with RIAs (CIS BIO International, F91192 Gif-Sur-Vvette Cedex, France; Diagnostic Products, Los Angeles, CA; and Insik-5 P2796 kit, Sorin Biomedica,
Italy, respectively). Plasma concentrations of nonesterified fatty acids (NEFA) (36), glycerol (5), acetocetate, and β-hydroxybutyrate (43, 44) were determined with enzymatic methods.

Calculations. Estimates of whole body leucine and glucose kinetics were made at steady-state conditions during 30 min on the evening before the study day (baseline period), during 30 min on the study day (experimental period), and during the end of the clamping period (270–300 min) on the study day. Total leucine flux was calculated by dividing the infusion rate of [1-13C]leucine by the α-[1-13C]KIC TTR according to the reciprocal pool model (6, 25). Leucine oxidation rate (representing irreversible leucine catabolism) was calculated by dividing the product of 13CO2 atom percent excess and VCO2 in expired air by plasma α-[1-13C]KIC TTR. A CO2 retention factor of 0.81 was used (1).

Infusion of glucose during glucose clamping with a different natural 13CO2 content may lead to an error of the calculated leucine oxidation rate by influencing the background 13CO2-to-12CO2 ratio. To address this question, Laager et al. (30) recently determined background 13C enrichment while infusing high doses of insulin and glucose but no 13C-leucine tracer during 8 h. They found a 6% overestimation of the leucine oxidation rate (30) during glucose infusion, a number which would be lower in the present study due to the lower glucose infusion rate (~40% of that studied by Laager et al.). Thus there was only a minimal effect of glucose infusions on calculated leucine oxidation rates during clamping in all protocols of the present study.

Nonoxidative leucine disappearance (representing whole body protein synthesis) was calculated by subtracting the rate of leucine oxidation from total leucine flux. Endogenous leucine flux (a parameter of protein breakdown) was calculated by subtracting the infusion rate of unlabeled leucine from total leucine flux. Net balance of leucine metabolism was calculated as the difference between the rate of nonoxidative disappearance and endogenous flux of leucine. Endogenous glucose rate of appearance (Ra) was calculated by dividing the [6,6-2H2]glucose infusion rate by plasma glucose TTR. During glucose clamping, total plasma glucose Ra was the sum of the glucose infusion rate and endogenous glucose Ra. Glucose Ra divided by the corresponding plasma glucose concentration yielded the glucose metabolic clearance rate (MCR).

Respiratory quotients were calculated by dividing VCO2 (ml/min) by VO2 (ml/min), where VO2 is oxygen consumption. Resting energy expenditures (kcal/24 h) and utilization of fat and carbohydrates as percentage of nonprotein energy expenditure were determined by indirect calorimetry (28).

Statistical analysis. Repeated-measures ANOVA of Statview and Student’s paired t-tests (Abacus Concepts, Berkeley, CA) on a Power Macintosh 7100/80 were used to detect differences between and within the three protocols. Bonferroni-Dunn and Scheffé’s F procedures were performed for correction of double comparisons. Statistical tests were only performed to assess differences between the hypoosmolality and isoosmolality studies and the hyperosmolality and isoosmolality studies and within all groups. Results are means ± SE.

RESULTS

Plasma sodium, osmolality, potassium, and water balance. Plasma sodium concentrations remained unchanged in the isoosmolality study (baseline values, 142 ± 0.4; end of study, 140 ± 0.6 mmol/l); they increased in the hyperosmolality study from 142 ± 0.2 to 149 ± 0.4 mmol/l (P < 0.0001) and decreased in the hypoosmolality study from 142 ± 0.4 to 131 ± 0.5 mmol/l (P < 0.0001) between 8 PM (end of baseline period) and 1 PM (end of the study) (Fig. 2). Osmolality (mmol/kg H2O) decreased slightly during the isoosmolality study from 286 ± 1 to 283 ± 1, increased during the hyperosmolality study from 283.4 ± 0.5 to 296.4 ± 0.7 (P < 0.0001), and decreased during the hypoosmolality study from 283 ± 0.4 to 265 ± 1 (P < 0.0001) between 8 PM (end of baseline period) and the end of the study at 1 PM on the next day. Plasma potassium concentrations (mmol/l) during the baseline period were 3.7 ± 0.4, 3.9 ± 0.6, and 3.8 ± 0.6 during the isoosmolality, hyperosmolality, and hypoosmolality studies, respectively. They remained unchanged until the end of the study in the hyperosmolality (3.9 ± 0.04) and isoosmolality (3.8 ± 0.04) studies and decreased in the hypoosmolality study to 3.6 ± 0.06 (P < 0.02 vs. baseline).

![Fig. 2. Sodium plasma concentrations (mmol/l) and plasma osmolality (mmol/kg H2O) in isoosmolality (G, middle curve), hyperosmolality (E, top curve), and hypoosmolality (C, bottom curve) studies. Data are means ± SE; n = 10 subjects/group.](http://apenda.physiology.org/Downloadedfrom)
Water balance in the isoosmolality study and the hyperosmolality study was zero; fluid balance increased in the hypoosmolality study by 2.01 ± 0.07 kg, as calculated from urinary output and water administration.

Leucine kinetics. TTR of α-KIC and of CO₂ reached nearly steady-state conditions during all three measurement periods in all three studies (Fig. 3). Figure 4 demonstrates that endogenous leucine flux (µmol·kg⁻¹·min⁻¹) decreased in the hypoosmolality study from the baseline to the experimental period of the next morning from 1.9 ± 0.05 to 1.79 ± 0.06 compared with the isoosmolality study (P < 0.02 vs. hypoosmolality, repeated-measures ANOVA) and remained unchanged in the isoosmolality and hyperosmolality studies (from 1.79 ± 0.09 to 1.88 ± 0.1 and from 1.8 ± 0.06 to 1.71 ± 0.06, respectively). During clamping endogenous leucine flux decreased to 1.4 ± 0.6, 1.31 ± 0.03, and 1.4 ± 0.04 in the isoosmolality study, and hypoosmolality studies, respectively (P < 0.0001 vs. experimental period for all groups), with no significant difference between them. Leucine oxidation decreased from baseline values to the experimental period from 0.34 ± 0.03 to 0.27 ± 0.01 µmol·kg⁻¹·min⁻¹ (P < 0.03 vs. baseline, P < 0.005 vs. isoosmolality, repeated-measures ANOVA) during the hypoosmolality study and remained unchanged in the isoosmolality study (0.31 ± 0.02 during the baseline period, 0.32 ± 0.02 during the experimental period) and in the hyperosmolality study (0.31 ± 0.02 and 0.31 ± 0.01, respectively). Leucine oxidation was significantly lower during the hypoosmolality study than during the isoosmolality study (P < 0.02 vs. isoosmolality, paired t-test). Leucine oxidation increased to 0.42 ± 0.02, 0.43 ± 0.01, and 0.41 ± 0.02 µmol·kg⁻¹·min⁻¹ in the isoosmolality, hyperosmolality, and hypoosmolality studies, respectively, during the clamping period (P < 0.0001 vs. experimental period for all groups). Nonoxidative leucine flux remained unchanged between the baseline and the experimental period of all studies. It increased during clamping in all studies (P < 0.0001 vs. experimental period), without difference between them. Plasma leucine concentrations were 153 ± 10, 157 ± 11, and 152 ± 5 µmol/l during the baseline period of the isoosmolality, hyperosmolality, and hypoosmolality studies, respectively, during the clamping period (P < 0.0001 vs. experimental period for all groups). Plasma leucine concentrations were 157 ± 10, 157 ± 11, and 152 ± 5 µmol/l during the baseline period of the isoosmolality, hyperosmolality, and hypoosmolality studies, respectively. They remained unchanged during the isoosmolality (145 ± 5) and hyperosmolality studies (147 ± 5) but decreased slightly during the hypoosmolality study to 143 ± 2 µmol/l (P < 0.05 vs. baseline values). During insulin-glucose-amino acid clamp plasma leucine concentrations were 149 ± 6, 149 ± 4, and 150 ± 3 µmol/l during the isoosmolality, hyperosmolality, and hypoosmolality studies, respectively (nonsignificant vs. experimental period).
Glucose kinetics. Plasma glucose concentrations were higher during the hyperosmolality study (P < 0.03, paired t-test) and lower during the hyposmolality study (P < 0.03 vs. isosmolality study) compared with the isosmolality study (Table 1). R_a decreased from baseline to the experimental period during the hyposmolality study (P < 0.02 paired t-test) and remained unchanged during the hyperosmolality and hyposmolality studies. Glucose R_s during the experimental period was higher in the hyposmolality study than in the isosmolality study (P < 0.005 paired t-test) and the increase in R_s during clamping was similarly decreased in all studies (P < 0.05 or less). Plasma insulin concentrations were lower during the experimental period of the hyposmolality study compared with the isosmolality study (P < 0.01 vs. isosmolality).

Plasma concentrations of insulin, C-peptide, glucagon, glycerol, NEFA, acetoacetate, and β-hydroxybutyrate and of antidiuretic hormone Plasma insulin concentrations were lower during the experimental period of the hyposmolality study compared with the isosmolality study (P < 0.05) (Table 2). Plasma insulin concentrations increased during clamping, and C-peptide decreased similarly in all studies (P < 0.05 or less). C-peptide concentrations during clamping were lower during the hyposmolality study than during the isosmolality study (P < 0.05). Glucagon plasma concentrations increased similarly during clamping in all studies (P < 0.05 or less). NEFA concentrations were slightly higher during the hyposmolality study compared with the isosmolality study (nonsignificant), and they decreased similarly during clamping in all studies (P < 0.005 or less). Plasma glycerol concentrations during the experimental period were higher during the hyposmolality study than during the isosmolality study (P < 0.05). Plasma glycerol concentrations were similarly decreased during clamping in all studies. Plasma acetoacetate concentrations were similarly decreased during clamping in all studies. Plasma acetoacetate concentrations were similarly decreased during clamping in all studies. Plasma acetoacetate concentrations were similarly increased during clamping.

<table>
<thead>
<tr>
<th>Table 2. Plasma concentrations of insulin, C-peptide, glucagon, NEFA, glycerol, acetoacetate, and β-hydroxybutyrate</th>
<th>Experimental</th>
<th>Clamping Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, pmol/l</td>
<td>40.8 ± 4.8</td>
<td>154.8 ± 4.8^a</td>
</tr>
<tr>
<td>C-peptide, nmol/l</td>
<td>0.378 ± 0.025</td>
<td>0.322 ± 0.027^b</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>74 ± 3</td>
<td>83 ± 5^b</td>
</tr>
<tr>
<td>NEFA, µmol/l</td>
<td>414 ± 17</td>
<td>241 ± 23^b</td>
</tr>
<tr>
<td>Glycerol, µmol/l</td>
<td>43 ± 0.003</td>
<td>18 ± 0.3</td>
</tr>
<tr>
<td>Acetoacetate, µmol/l</td>
<td>42 ± 5</td>
<td>30 ± 2^c</td>
</tr>
<tr>
<td>β-Hydroxybutyrate, µmol/l</td>
<td>66 ± 13</td>
<td>13 ± 5</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 10/group. NEFA, nonesterified fatty acids. ^aP < 0.05 vs. isosmolality (paired t-tests); ^bP < 0.05, ^P < 0.005 vs. experimental period (paired t-tests).

Table 1. Glucose plasma concentrations and kinetics

<table>
<thead>
<tr>
<th>Iosmosmolality study</th>
<th>Baseline</th>
<th>Experimental</th>
<th>Clamping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose plasma concentration, mmol/l</td>
<td>4.8 ± 0.2</td>
<td>4.9 ± 0.1</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>Glucose MCR, ml·kg⁻¹·min⁻¹</td>
<td>2.8 ± 0.1</td>
<td>2.3 ± 0.1^c</td>
<td>4.4 ± 0.4^d</td>
</tr>
<tr>
<td>Endogenous glucose R_s, µmol·kg⁻¹·min⁻¹</td>
<td>13 ± 0.7</td>
<td>11.2 ± 0.4^e</td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>Hypersmolality study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose plasma concentrations, mmol/l</td>
<td>4.9 ± 0.05</td>
<td>5.1 ± 0.05^a</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>Glucose MCR, ml·kg⁻¹·min⁻¹</td>
<td>2.8 ± 0.1</td>
<td>2.5 ± 0.1^f</td>
<td>4.2 ± 0.4^d</td>
</tr>
<tr>
<td>Endogenous glucose R_s, µmol·kg⁻¹·min⁻¹</td>
<td>13.5 ± 0.6</td>
<td>12.4 ± 0.4^g</td>
<td>6 ± 0.6</td>
</tr>
<tr>
<td>Hypersmolality study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose plasma concentrations, mmol/l</td>
<td>4.8 ± 0.1</td>
<td>4.7 ± 0.05^a</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>Glucose MCR, ml·kg⁻¹·min⁻¹</td>
<td>2.7 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>3.3 ± 0.2^d</td>
</tr>
<tr>
<td>Endogenous glucose R_s, µmol·kg⁻¹·min⁻¹</td>
<td>12.8 ± 0.1</td>
<td>11.1 ± 0.3</td>
<td>4.6 ± 0.6</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 10 subjects/group. MCR, metabolic clearance rate; R_a, rate of appearance; ^aP < 0.05 vs. isosmolality (paired t-tests); ^bP < 0.05 vs. isosmolality (repeated-measures ANOVA); ^P < 0.05 vs. baseline (paired t-tests); ^dP < 0.005 vs. experimental (paired t-tests).
Because of crossreactivity of the administered vasopres-
sin analog Minirin with antidiuretic hormone, plasma
antidiuretic hormone concentrations were not mea-
sured under hypoosmolar conditions.

Indirect calorimetry. Resting energy expenditure de-
creased from baseline to the experimental period dur-
ing the isoosmolality (P < 0.05) and hypoosmolality
studies (P < 0.005 vs. baseline) but remained un-
changed during the hyperosmolality study (Table 3).

Table 3. Resting energy expenditure, respiratory gas
exchange respiratory quotient, and utilization of
carbohydrates and fat as percentage of nonprotein
energy expenditure

<table>
<thead>
<tr>
<th>Isosmolality study</th>
<th>Baseline</th>
<th>Experimental</th>
<th>Clamping Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>REE, kcal/24 h</td>
<td>1,724 ± 73</td>
<td>1,590 ± 65</td>
<td>1,651 ± 70</td>
</tr>
<tr>
<td>VO₂, ml/min</td>
<td>247 ± 12</td>
<td>228 ± 9</td>
<td>235 ± 11</td>
</tr>
<tr>
<td>VCO₂, ml/min</td>
<td>221 ± 9</td>
<td>207 ± 8</td>
<td>219 ± 11e</td>
</tr>
<tr>
<td>RQ</td>
<td>0.91 ± 0.02</td>
<td>0.90 ± 0.02</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td>C%</td>
<td>64 ± 5</td>
<td>73 ± 5</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>F%</td>
<td>36 ± 5</td>
<td>27 ± 5</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>Hyperosmolality study</td>
<td>1,729 ± 71</td>
<td>1,668 ± 90</td>
<td>1,776 ± 77</td>
</tr>
<tr>
<td>VO₂, ml/min</td>
<td>251 ± 12</td>
<td>242 ± 14</td>
<td>248 ± 13</td>
</tr>
<tr>
<td>VCO₂, ml/min</td>
<td>214 ± 7</td>
<td>207 ± 10</td>
<td>224 ± 8</td>
</tr>
<tr>
<td>C%</td>
<td>86 ± 0.03</td>
<td>86 ± 0.02</td>
<td>91 ± 0.03</td>
</tr>
<tr>
<td>F%</td>
<td>64 ± 8</td>
<td>50 ± 6</td>
<td>67 ± 9e</td>
</tr>
<tr>
<td>Hypoosmolality study</td>
<td>1,813 ± 99</td>
<td>1,583 ± 83d</td>
<td>1,691 ± 92e</td>
</tr>
<tr>
<td>VO₂, ml/min</td>
<td>263 ± 15</td>
<td>232 ± 13f</td>
<td>245 ± 12</td>
</tr>
<tr>
<td>VCO₂, ml/min</td>
<td>223 ± 10</td>
<td>189 ± 8 d</td>
<td>212 ± 11f</td>
</tr>
<tr>
<td>RQ</td>
<td>0.85 ± 0.02</td>
<td>0.82 ± 0.03</td>
<td>0.87 ± 0.02</td>
</tr>
<tr>
<td>C%</td>
<td>53 ± 8</td>
<td>43 ± 9</td>
<td>51 ± 6e</td>
</tr>
<tr>
<td>F%</td>
<td>47 ± 8</td>
<td>57 ± 9</td>
<td>49 ± 6e</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 10 subjects/group. REE, resting energy expenditure; RQ, respiratory gas exchange respiratory quotient; C% and F%, utilization of carbohydrates and fat as percentage of nonprotein energy expenditure, respectively; VO₂ and VCO₂, oxygen consumption and carbon dioxide production rates, respectively; P < 0.05 vs. isosmolality (paired t-tests); P < 0.05 vs. isoosmolality (repeated-measures ANOVA); P < 0.05 vs. isosmolality (paired t-tests); P < 0.05, P < 0.005 vs. Baseline (paired t-tests); P < 0.05, P < 0.005 vs. Experimental (paired t-tests).

DISCUSSION

The present study examined for the first time the
effect of acute hyperosmolality and hypoosmolality on
whole body protein and glucose metabolism in humans.
Administration of a vasopressin analog and liberal
water drinking, on the one hand, or infusion of a
hypertonic saline solution and restriction from drink-
ing, on the other hand, were used to produce changes of

Endogenous glucose Ra (representing mainly hepatic
protein breakdown) and glucose concentrations were
increased during hyperosmolality compared with isoosmolality. In contrast, plasma glucose concentra-
tions were decreased in the hypoosmolality study com-
pared with the isosmolality study. The increase in
glucose metabolic clearance rate during clamping was
diminished during hyperosmolar conditions, indicating
diminished insulin sensitivity of peripheral glucose
metabolism.

Concerning the effects of hypoosmolality, these data
should be discussed in the light of previous findings
obtained in vitro, suggesting that short-term modula-
tion of cell volume within a narrow range acts as a
potent signal to modify cellular metabolism and gene
expression (20). Hypoosmolal liver cell swelling in-
creases protein synthesis, glycogen synthesis, and amino
acid uptake and decreases proteolysis, glycogenolysis,
and glycolysis, whereas opposite metabolic effects are
triggered by cell shrinkage (18, 20, 41). It has been
demonstrated that in isolated perfused livers and hepa-
tocytes, insulin increases and glucagon decreases cellu-
lar volume within minutes (13), and these effects may
explain in part their metabolic effects. The Na+-
dependent amino acid transport systems in the plasma
membrane may act as a transmembrane signaling
system, triggering cellular function by altering cellular
hydridation in response to substrate delivery (18–20).
Regulation of phosphatidylinositol 3-kinase in skeletal
muscle may be an important component of the signal-
ing mechanisms involved in cell volume-mediated con-
trol of membrane transport (34).

The present finding may be important to understand
the pathogenesis of protein catabolism in various dis-

diseases. Indeed, a close relationship between cellular
hydridation of skeletal muscle and nitrogen balance has
been demonstrated in severely ill patients (21). Regard-
ing the question whether the observed metabolic effects
of changes in osmolality are in fact related to changes in
cell volume, no direct measurements of cell volume

Because of crossreactivity of the administered vasopres-
sin analog Minirin with antidiuretic hormone, plasma
antidiuretic hormone concentrations were not mea-
sured under hypoosmolar conditions.

Indirect calorimetry. Resting energy expenditure de-
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energy expenditure

<table>
<thead>
<tr>
<th>Isosmolality study</th>
<th>Baseline</th>
<th>Experimental</th>
<th>Clamping Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>REE, kcal/24 h</td>
<td>1,724 ± 73</td>
<td>1,590 ± 65</td>
<td>1,651 ± 70</td>
</tr>
<tr>
<td>VO₂, ml/min</td>
<td>247 ± 12</td>
<td>228 ± 9</td>
<td>235 ± 11</td>
</tr>
<tr>
<td>VCO₂, ml/min</td>
<td>221 ± 9</td>
<td>207 ± 8</td>
<td>219 ± 11e</td>
</tr>
<tr>
<td>RQ</td>
<td>0.91 ± 0.02</td>
<td>0.90 ± 0.02</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td>C%</td>
<td>64 ± 5</td>
<td>73 ± 5</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>F%</td>
<td>36 ± 5</td>
<td>27 ± 5</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>Hyperosmolality study</td>
<td>1,729 ± 71</td>
<td>1,668 ± 90</td>
<td>1,776 ± 77</td>
</tr>
<tr>
<td>VO₂, ml/min</td>
<td>251 ± 12</td>
<td>242 ± 14</td>
<td>248 ± 13</td>
</tr>
<tr>
<td>VCO₂, ml/min</td>
<td>214 ± 7</td>
<td>207 ± 10</td>
<td>224 ± 8</td>
</tr>
<tr>
<td>C%</td>
<td>86 ± 0.03</td>
<td>86 ± 0.02</td>
<td>91 ± 0.03</td>
</tr>
<tr>
<td>F%</td>
<td>64 ± 8</td>
<td>50 ± 6</td>
<td>67 ± 9e</td>
</tr>
<tr>
<td>Hypoosmolality study</td>
<td>1,813 ± 99</td>
<td>1,583 ± 83d</td>
<td>1,691 ± 92e</td>
</tr>
<tr>
<td>VO₂, ml/min</td>
<td>263 ± 15</td>
<td>232 ± 13f</td>
<td>245 ± 12</td>
</tr>
<tr>
<td>VCO₂, ml/min</td>
<td>223 ± 10</td>
<td>189 ± 8 d</td>
<td>212 ± 11f</td>
</tr>
<tr>
<td>RQ</td>
<td>0.85 ± 0.02</td>
<td>0.82 ± 0.03</td>
<td>0.87 ± 0.02</td>
</tr>
<tr>
<td>C%</td>
<td>53 ± 8</td>
<td>43 ± 9</td>
<td>51 ± 6e</td>
</tr>
<tr>
<td>F%</td>
<td>47 ± 8</td>
<td>57 ± 9</td>
<td>49 ± 6e</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 10 subjects/group. REE, resting energy expenditure; RQ, respiratory gas exchange respiratory quotient; C% and F%, utilization of carbohydrates and fat as percentage of nonprotein energy expenditure, respectively; VO₂ and VCO₂, oxygen consumption and carbon dioxide production rates, respectively; P < 0.05 vs. isosmolality (paired t-tests); P < 0.05 vs. isoosmolality (repeated-measures ANOVA); P < 0.05 vs. isosmolality (paired t-tests); P < 0.05, P < 0.005 vs. Baseline (paired t-tests); P < 0.05, P < 0.005 vs. Experimental (paired t-tests).
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could be obtained in our study. In vitro regulatory mechanisms are activated within minutes in response to hypoosmolality or hyperosmolality exposure. However, these volume-regulatory mechanisms do not completely restore cell hydration. This has been studied extensively in liver (11, 22, 42). In fact, a decrease of extracellular osmolality in perfused rat livers by 20 mosmol/kgH₂O resulted in a persistent 4.7 ± 1.3% increase in cell hydration (S. von Dahl and D. Häussinger, unpublished data). The extent of this cell volume deviation after completion of volume regulatory ion fluxes determines the metabolic changes (14, 20, 31) that persist as long as the anisotonicity is maintained. The longest time period studied in vitro was about 2 h. Therefore, we believe that alterations of cell volume were likely to explain the present findings. The present alterations may be comparable to those in vitro from above. However, such small changes of cell volume are difficult to measure with sufficient precision.

The reason that acute hyperosmolarity did not result in protein catabolism in the present study is not obvious; it is possible that 17 h was an inappropriate time frame over which to detect any changes in protein breakdown and/or that the increase in plasma osmolality was too modest to exert sufficient cell shrinking. However, more pronounced experimental dehydration in living human volunteers would be too risky. It also should be noted that net water balance was only affected in the hypoosmolality group, whereas the hyperosmolality group experienced no change of water balance.

Postabsorptive plasma insulin concentrations were lower during hypoosmolality, and C-peptide suppression during clamping was increased. Compared with isoosmolality, this is a yet undescribed phenomenon. The antiproteolytic effect of insulin is well described (10); therefore, lowered insulin levels during hypoosmolal conditions may partly counteract the antiproteolytic effect in humans, thereby masking, in part, the antiprotein catabolic effects of hypoosmolality. On the other hand, decreased insulin concentrations may contribute to the observed increase in fat utilization as observed by indirect calorimetry and to the increase in plasma glycerol concentrations. Increased oxidation of lipid substrates and increased ketone body concentrations may explain, in part, the protein-sparing effect during hypoosmolality (38). Additionally, the increase in glycerol concentrations during hypoosmolal conditions may exert a nitrogen-sparing effect by conserving amino acids as gluconeogenic precursors, thus making amino acids available for reincorporating into protein and reducing urea production (26). The mechanism by which hypoosmolality increased lipolysis remained unclear; plasma catecholamines were not increased during hypoosmolality (S. Bilza and U. Keller, unpublished data). Oxidation of carbohydrates was decreased in the present study. Several reasons may explain this finding. Increased utilization of fat reduces the need for glucose as a fuel (8). This may be the result of decreased plasma insulin levels, insulin being a potent inhibitor of lipolysis (27). When lipolysis is increased, more fatty acids are available for oxidation, and fatty acid oxidation is inversely related to plasma glucose concentrations (40).

It is concluded that moderate states of hyperosmolality and hypoosmolality influence protein, glucose, and fat metabolism; these effects may be linked to hypoosmolar cell swelling and hyperosmolar cell shrinkage. Hypoosmolality in the present study exerted a protein- and glucose-sparing effect with increased utilization of fat. In contrast, hyperosmolality exerted opposite effects on glucose metabolism with increased hepatic glucose production, resulting in modestly increased plasma glucose concentrations.

The results of the study therefore suggest that alterations in whole body water and ionic balance exert metabolic effects, which may be important in clinical situations of altered extracellular osmolality.

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Address for reprint requests: U. Keller, Deps. of Research and Internal Medicine, Univ. Hospital Basel, Petersgraben 4, 4031 Basel, Switzerland.

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REFERENCES
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