

Effects of Hypoosmolality on Whole-Body Lipolysis in Man

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Changes in extracellular osmolality, and thus in the cellular hydration state, appear to directly influence cell metabolism. The metabolic changes associated with cell swelling are inhibition of glycogenolysis, glycolysis, and proteolysis. Recent studies in our laboratory demonstrated diminished whole-body protein breakdown in humans during an acute hypoosmolar state. Because of the close interrelationship between carbohydrate and fat metabolism, we speculated that adipose tissue lipolysis and fatty acid oxidation are regulated by changes in extracellular osmolality. Therefore, we investigated the effect of artificially induced hypoosmolality on whole-body lipolysis and fat oxidation in seven healthy young men. Hypoosmolality was induced by intravenous administration of desmopressin, liberal ingestion of water, and infusion of hypotonic (0.45%) saline solution. Lipolysis was assessed by a stable-isotope method (2-[¹³C]-glycerol infusion). The glycerol rate of appearance (Ra), reflecting whole-body lipolysis, was higher under hypoosmolar compared with isoosmolar conditions (2.35 ± 0.40 v 1.68 ± 0.21 $\mu\text{mol/kg/min}$, $P = .03$). This was even more pronounced when lipolysis was suppressed during hyperinsulinemia and euglycemic clamping (0.90 ± 0.08 v 0.61 ± 0.03 $\mu\text{mol/kg/min}$, $P = .002$). However, plasma free fatty acid (FFA), glycerol, ketone body, insulin, and glucagon concentrations and carbohydrate and lipid oxidation measured by indirect calorimetry were not significantly altered by hypoosmolality. Plasma norepinephrine concentrations were lower under hypoosmolar conditions ($P < .01$ v control). In conclusion, hypoosmolality in vivo results in increased whole-body lipolysis, which is not due to changes in major lipolysis regulating hormones.

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LIPOLYSIS refers to a process in which triglycerides are hydrolyzed to glycerol and free fatty acids (FFAs). The majority of lipolysis occurs in adipose tissue; however, lipolysis in liver and muscle has considerable importance in compensating for short-term changes of the FFA supply and demand for lipid oxidation.¹ The activity of adipose tissue hormone-sensitive lipase HSL is the rate-limiting step for the mobilization of triglycerides from adipose tissue, and in the postabsorptive state most detectable lipolysis is mediated by this enzyme.^{1,2} A wide range of circulating hormones and other substrates, among which insulin and catecholamines are the most important, have been recognized as regulators of this enzyme.³

On the other hand, modulation of cell volume has been shown to act as a potent signal that modifies cellular metabolism.^{4,5} Both hormone- or substrate-induced changes in the activity of ion transport systems and direct exposure to anisoosmotic media resulted in altered cell size.⁶ Indeed, insulin has been shown to increase cell size in perfused liver and isolated hepatocytes, whereas glucagon exerted an opposite effect.⁵ Therefore, it has been suggested that changes of cell volume in response to hormones trigger certain patterns of cell function.⁶ Moreover, in vitro induced hypoosmotic liver cell swelling resulted in increased protein and glycogen synthesis, whereas proteolysis, glycogenolysis, and glycolysis decreased.^{5,7}

Recently, a study in human subjects in our laboratory demonstrated that protein breakdown and oxidation and hepatic glucose production decreased after induction of an acute state of hypoosmolality.⁸ Whether FFA metabolism, ie, lipolysis and

lipid oxidation, are similarly influenced by changes of extracellular osmolality has not been investigated previously. Therefore, we measured whole-body lipolysis and lipid oxidation under hypoosmolar conditions in vivo in human subjects using a stable-isotope method and indirect calorimetry.

SUBJECTS AND METHODS

Subjects

Seven healthy young men aged 24 ± 2 years (mean \pm SEM) with a body mass index of 22 ± 1.6 kg/m^2 participated in the study. All subjects were in good health and none were taking any medication. Their medical history was unremarkable, and a physical examination and standard laboratory tests before beginning the study did not reveal any abnormalities. Written informed consent was obtained after explanation of the nature, purpose, and potential risks of the study. The study protocol was approved by the ethics committee of the Department of Internal Medicine of the University Hospital Basel, Switzerland.

Experimental Design

Each subject was studied twice (control study and hypoosmolality study) with an interval of at least 1 week in randomized order. At 4:30 PM of the first day, all subjects were admitted to the metabolic study unit of the hospital after fasting since 11:00 AM. A polyethylene cannula was placed into the right antecubital vein for infusions, and a 21-gauge butterfly needle was inserted in a retrograde manner in a dorsal superficial vein of the right hand. The hand was kept at 55°C in a thermostat-controlled chamber to obtain arterialized blood samples as described previously.⁹ At 5:00 PM, after obtaining background blood samples, 2-[¹³C]-glycerol (99% enriched, sterile, and pyrogen-free; Mass Trace, Somerville, MA) dissolved in normal saline, with a priming dose of 1.125 $\mu\text{mol/kg}$ followed by continuous infusion at a rate of 0.075 $\mu\text{mol/kg/min}$, was infused for 150 minutes. During the last 30 minutes, three blood samples were obtained at 15-minute intervals. Thereafter, at 8:00 PM, a standard meal containing 600 kcal was served, and all subjects remained fasting until the end of the study on the following day. A second 2-[¹³C]-glycerol infusion for 270 minutes was started at 8:00 AM of the next day (Fig 1).

During the hypoosmolality study, 4 μg Minirin (desmopressin; Ferring, Dübendorf, Switzerland) was administered intravenously at 8:00 PM of the first day, and the subjects were instructed to consume 2.5 to 3 L tap water overnight. At 8:00 AM the following day, 4 μg Minirin

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Submitted April 24, 1998; accepted October 22, 1998.

Supported by Grant No. 32-39747.93 from the Swiss National Science Foundation, and by the "Wissenschaftlicher Kredit" of the Kantonsspital Basel.

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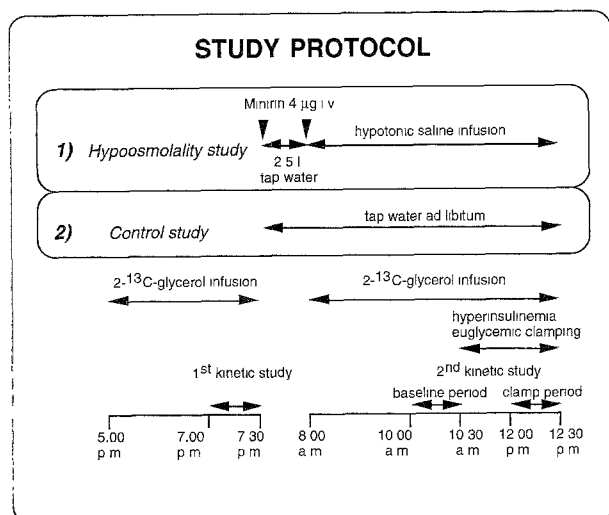


Fig 1. Study protocol. Seven human subjects were studied in random order during hypoosmolality and isoosmolality (control study). The 1st kinetic study (before intervention) was performed on day 1 in the evening, and the 2nd kinetic study on day 2 in the morning (after intervention).

was administered and an infusion of hypotonic saline (0.45%) was started at a rate of 200 mL/h and continued until the end of the second 2-[¹³C]-glycerol infusion. Three blood samples were obtained at 15-minute intervals after 120 minutes and during the last 30 minutes of euglycemic clamping. During the control study, Minirin and hypotonic saline were not used, and the subjects were allowed to consume tap water ad libitum.

Euglycemic-Hyperinsulinemic Clamp

During the last 120 minutes of the second 2-[¹³C]-glycerol infusion, a euglycemic-hyperinsulinemic clamp study was performed. A continuous infusion of insulin 60 mU/m²/min (Insulin Actrapid HM; Novo Nordisk, K  nsnacht, Switzerland) was administered during the initial 3 minutes of the clamp, and the infusion rate was reduced to 15 mU/m²/min during the following 117 minutes. Glucose 20% was infused at variable rates to maintain euglycemia, and the glucose infusion rate was adjusted every 5 to 10 minutes according to the rapidly measured plasma glucose concentration (YSI Glucose Analyzer; YSI, Yellow Springs, OH). A standard amino acid solution (Vamina 10%; Pharmacia & Upjohn, D  bendorf, Switzerland) was administered at the same time at a rate of 0.0144 mg/kg/min to prevent an insulin-induced decrease of plasma amino acid concentrations.¹⁰

Indirect Calorimetry

Indirect calorimetry was performed to measure carbohydrate and fat oxidation using a ventilated-hood metabolic monitor (Deltatrac II MBM-200; Datex, Helsinki, Finland) during the first kinetic study and the baseline and clamp period of the second kinetic study. Expired air was collected for 20 minutes at each time point.

Analytical Methods

The plasma was immediately separated by centrifugation at 4,000 rpm at 4  C and stored in part at -70  C for later analysis. Plasma sodium and osmolality were analyzed immediately by potentiometry (Dupont Dimension AR; Dade, D  dingen, Switzerland) and a cryoscopic technique (Micro Osmometer 3 MO; Advanced Instruments, Norwood, MA), respectively. The plasma tracer to tracee ratio (TTR) of 2-[¹³C]-glycerol was measured by gas chromatography-mass spectrom-

etry (model 5890/5790; Hewlett-Packard, Palo Alto, CA).¹¹ Plasma concentrations of glycerol,¹² FFA,¹³ acetoacetate, and β -hydroxybutyrate^{14,15} were determined using enzymatic methods. Plasma concentrations of C-peptide,¹⁶ glucagon, and insulin were measured using radioimmunoassays. Plasma triglyceride concentrations were measured using enzymatic methods (Boehringer Mannheim, Rothrist, Switzerland). Plasma catecholamines were measured using a high-performance liquid chromatographic method¹⁷ (Laboratorium Dr. Viollier, Allschwil, Switzerland).

Calculations and Statistics

Since stable levels of the plasma glycerol concentration and enrichment were obtained, the glycerol rate of appearance (Ra) was calculated assuming steady-state conditions by dividing the infusion rate of 2-[¹³C]-glycerol by the glycerol TTR in plasma.¹⁸ The TTR used in the calculations was the mean of three consecutive plasma samples obtained during the kinetic studies and clamp periods, respectively. Likewise, the plasma concentrations of glycerol, FFA, acetoacetate, β -hydroxybutyrate, insulin, C-peptide, and glucagon were the means of three consecutive measurements of samples obtained at the same time points. Respiratory quotients (RQs) were calculated by dividing CO₂ production by O₂ consumption. Values for carbohydrate and fat oxidation, nonprotein RQs, and energy expenditure were calculated using standard formulas assuming a nitrogen excretion rate of 13 g/d.¹⁹

All data are expressed as the mean \pm SEM. Statistical analyses were performed using Student's paired *t* test (StatView; Abacus Concepts, Berkeley, CA) on a MacIntosh Performa 5400/160 computer (Apple Computer, Cupertino, CA).

RESULTS

Plasma Sodium and Osmolality

Plasma sodium concentrations remained unchanged during the control study (first kinetic study, 142 ± 0.4 mmol/L; second kinetic study; baseline period, 141 ± 0.6 mmol/L; clamp period, 141 ± 0.5 mmol/L). During the hypoosmolality study, the levels decreased from 142 ± 0.4 mmol/L (first kinetic study) to 132 ± 0.6 mmol/L (second kinetic study, baseline period, $P < .0001$) and 130 ± 0.4 mmol/L (second kinetic study, clamp period, $P < .0001$), respectively. In analogy, plasma osmolality remained unchanged during the control study (first kinetic study, 285 ± 1.3 ; second kinetic study; baseline period, 283 ± 1.3 milliosmoles [mOsm]/kg H₂O; clamp period, 283 ± 1.4 mOsm/kg H₂O) but decreased during the hypoosmolality study (first kinetic study, 286 ± 1.2 mOsm/kg H₂O; second kinetic study; baseline period, 266 ± 1.2 mOsm/kg H₂O; clamp period, 265 ± 0.8 mOsm/kg H₂O, $P < .0001$). Both plasma sodium and plasma osmolality were lower during the second kinetic study (baseline period, $P = .0002$; clamp period, $P < .0001$) of the hypoosmolality study.

Glycerol Kinetics

The glycerol Ra was similar during the first kinetic study of the control study (2.12 ± 0.18 μ mol/kg/min) and hypoosmolality study (2.17 ± 0.22 μ mol/kg/min). During the baseline period of the second kinetic study (after a 14-hour fast, at 10 AM), the glycerol Ra was higher during hypoosmolar versus control conditions (2.35 ± 0.40 v 1.68 ± 0.21 μ mol/kg/min, $P = .03$). This difference was also apparent during the clamp period (0.90 ± 0.08 v 0.61 ± 0.03 μ mol/kg/min, $P = .002$). Compared with the baseline period, the glycerol Ra was lower

during the clamp period of both the control study and hypoosmolality study ($P < .01$) (Fig 2).

Plasma Glycerol, FFA, Ketone Bodies, Insulin, C-Peptide, Glucagon, Triglycerides, and Catecholamines

There were no significant differences in plasma glycerol, FFA, ketone body, insulin, C-peptide, and glucagon concentrations between the hypoosmolality and control studies. Plasma glycerol and FFA concentrations were decreased during the clamp period of the second kinetic studies ($P < .0001$ v baseline). Equally, ketone bodies decreased during the clamp period of both studies ($P < .01$ and $P < .02$ for acetoacetate and β -hydroxybutyrate, respectively). Plasma insulin increased to a similar extent during the clamp periods ($P < .0002$). C-peptide concentrations were suppressed during the clamp periods ($P < .0002$). Glucagon increased during the clamp periods ($P = .01$). When these changes during the clamp periods of both protocols were compared, there was no difference for any of the parameters (Table 1).

Plasma triglyceride concentrations were not different between the baseline and clamp periods of both the hypoosmolality and control studies. When triglyceride concentrations from the baseline and clamp studies were compared, there was no difference during the control study. However, during the hypoosmolality study, triglycerides were significantly decreased during clamping (Table 1).

Plasma epinephrine concentrations did not differ between the baseline and clamp periods of the second kinetic studies when the hypoosmolality and control study were compared. Plasma norepinephrine concentrations were lower during both the baseline period ($P = .002$ v control) and the clamp period ($P = .01$ v control) of the second kinetic study of the hypoosmolality study, but showed no changes within both protocols (Table 1).

Indirect Calorimetry

Carbon dioxide production rates increased during the clamp periods of both studies ($P < .01$ v baseline), without a difference between the two studies. The oxygen production rate and resting energy expenditure remained stable within both studies, without a significant difference between the studies. RQs and

nonprotein RQs increased during the clamp periods of both protocols ($P \leq .05$). Carbohydrate utilization increased and fat utilization decreased during both clamp periods ($P \leq .01$). No difference was observed between the studies (Table 2).

DISCUSSION

Adipose tissue lipolysis by hormone-sensitive lipase is mainly regulated by insulin, glucagon, and catecholamines.³ Changes in cell size secondary to altered ion exchange through the cell membrane appear to serve as a mediator of the metabolic effects of these hormones.⁵ Insulin has been shown to increase liver cell volume and thereby to inhibit proteolysis, glycogenolysis, and glycolysis, whereas protein synthesis and glycogen synthesis are increased.⁶ Glucagon exerted opposite effects on the cell volume and metabolism, and anisoosmotic changes in cell size mimicked the metabolic actions of these hormones.^{6,20} Since these hormones are the main regulators of lipolysis³ and since there are close interrelations between fatty acid and glucose metabolism,²¹ it is tempting to speculate that hypoosmolality influences fatty acid metabolism in humans. Therefore, this study examined for the first time whether fatty acid metabolism is at least partially regulated by changes in plasma osmolality in humans.

Hypoosmolality was induced by liberal consumption of water, infusion of hypotonic saline, and administration of a vasopressin analog intravenously. The degree of hypoosmolality achieved in this study is likely to result in a mild degree of cell swelling. The key finding was an increase in the glycerol Ra in the postabsorptive state under hypoosmolar conditions compared against the control study. This difference was equally pronounced during hyperinsulinemic-euglycemic clamping, when the glycerol Ra decreased significantly under both hypotonic and isotonic conditions. Since the glycerol Ra is regarded as a true measure of whole-body lipolysis, these results suggest that hypoosmolality increases lipolysis *in vivo*. Plasma insulin, C-peptide, glucagon, and epinephrine concentrations were not different between the protocols. Therefore, hypoosmolality-induced changes in the concentration of these main regulators of adipose tissue lipolysis are not likely to account for the increased lipolytic rate under hypoosmolar conditions. However, plasma norepinephrine concentrations were lower during the hypoosmolality study. This suggests that the lipolytic effect of hypoosmolality was even underestimated due to the known lipolytic properties of norepinephrine.²²

To make these conclusions, one must be sure that the agent or approach to induce hypoosmolality does not stimulate lipolysis even in the absence of hypoosmolality. Although arginine-vasopressin has been reported to affect intermediary metabolism,^{23,24} the data concerning lipolytic effects of the hormone are contradictory, with reports of increased,²⁵⁻²⁷ decreased,²⁸ or unchanged²⁹ lipolysis. However, desmopressin (1-desamino-8-D-arginine-vasopressin), used in this study, is a highly selective agonist of the adenylate cyclase-coupled V2 receptor located at the basolateral membrane of the collecting duct cells in the kidney³⁰ and possibly the V1b receptor in the pineal gland.³¹ Thus, only arginine-vasopressin, not desmopressin, stimulated lipolysis in rabbit hearts, indicating that desmopressin itself could not explain our finding of increased lipolysis.²⁶ Furthermore, isotonic volume expansion in the absence of hypoosmo-

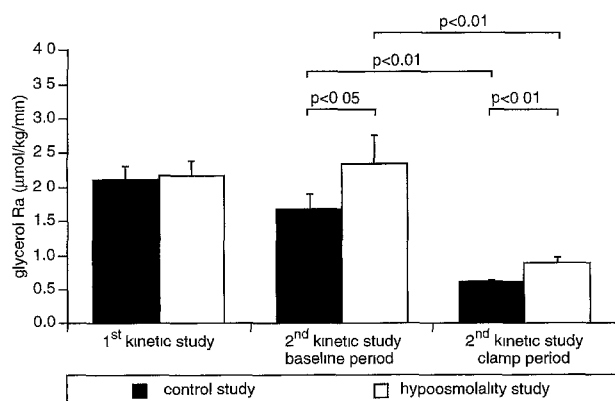


Fig 2. Glycerol Ra before (1st kinetic study) and during (2nd kinetic study) isoosmolality (control study) and hypoosmolality. Results are the mean \pm SEM.

Table 1. Glycerol Ra and Plasma Hormones and Substrates During the Second Kinetic Study

Parameter	Control Study		Hypoosmolality Study	
	Baseline	Clamp	Baseline	Clamp
Glycerol Ra ($\mu\text{mol/kg/min}$)	1.68 ± 0.21	0.61 ± 0.03	$2.35 \pm 0.40^{\S}$	$0.90 \pm 0.08^{\#}$
Glycerol ($\mu\text{mol/L}$)	49.4 ± 4.3	$12.7 \pm 1.5^*$	53.1 ± 5.0	$13.5 \pm 1.6^*$
FFA ($\mu\text{mol/L}$)	553 ± 38	$239 \pm 22^*$	543 ± 39	$249 \pm 23^*$
Acetoacetate ($\mu\text{mol/L}$)	105 ± 17	$43 \pm 4^{\dagger}$	101 ± 21	$37 \pm 3^{\dagger}$
β -Hydroxybutyrate ($\mu\text{mol/L}$)	170 ± 40	$23 \pm 3^*$	203 ± 46	$22 \pm 2^*$
Insulin ($\mu\text{U/L}$)	4.7 ± 0.4	$36.2 \pm 4.1^*$	3.6 ± 0.4	$36.0 \pm 1.8^*$
C-peptide (pmol/L)	415 ± 35	$158 \pm 23^*$	369 ± 37	$164 \pm 27^*$
Glucagon (pg/mL)	57.2 ± 1.2	$93.7 \pm 9.9^{\dagger}$	56.0 ± 2.9	$78.8 \pm 5.8^*$
Triglycerides (mmol/L)	0.58 ± 0.08	0.52 ± 0.06	0.57 ± 0.07	$0.39 \pm 0.07^*$
Epinephrine (pmol/L)	560 ± 102	649 ± 101	473 ± 123	564 ± 66
Norepinephrine (pmol/L)	$1,473 \pm 223$	$1,580 \pm 296$	$1,092 \pm 200^{\dagger}$	$1,093 \pm 244^{\S}$

* $P < .01$, $^{\dagger}P < .05$ v baseline. $^{\#}P < .01$, $^{\S}P < .05$ v control.

lality could enhance lipolysis; however, it has recently been shown that isotonic plasma volume expansion by 14% and 21%, respectively, using colloid infusions even decreased exercise-induced lipolysis assessed by measurement of the glycerol Ra.³² Therefore, it seems unlikely that the use of either desmopressin or isotonic volume expansion in the absence of hypoosmolality explained the present finding of increased lipolysis.

Glycerol released in significant amounts into the splanchnic circulation by visceral adipose tissue lipolysis and taken up by the liver would not be evident by measurement of the systemic glycerol Ra, due to a large instantaneous hepatic uptake of glycerol exceeding 80%.³³⁻³⁵ Consequently, a decrease of the hepatic fractional uptake of glycerol could increase the systemic glycerol Ra secondary to a relative increase in hepatic glycerol output despite unchanged adipose tissue lipolysis. However, the finding of negligible net gut glycerol output in resting dogs³⁶ and human subjects³⁷ led several investigators to conclude that an underestimation of whole-body lipolysis as measured by the glycerol Ra due to hepatic first-pass clearance of glycerol released from the gut is not likely to occur.^{38,39} Taken together, a diminished hepatic extraction of glycerol during hypoosmolality may partially explain the present findings, but the magnitude of increase (40%) in the glycerol Ra suggests an additional increase in whole-body lipolysis.

Plasma glycerol and FFA concentrations were not significantly affected by hypoosmolality. However, although the concentrations of these metabolites may reflect qualitative changes in lipolysis, they should not be regarded as a quantitative representation of lipolytic activity, because of the nonlinear relationship between turnover and concentration.⁴⁰ In addition,

the primary reesterification of FFAs before their release from adipose tissue, as indicated by a ratio of the FFA Ra to glycerol Ra less than 3:1,^{38,41} could explain the present finding of unchanged plasma FFA concentrations even in the presence of an increase in the glycerol Ra. However, recent studies using stable isotopes⁴² and measuring glycerol and FFA release from adipose tissue⁴³ suggest that fatty acid reesterification may not occur in the postabsorptive state.

Although lipolysis in the fasting state has been reported to be mainly due to the action of adipose tissue hormone-sensitive lipase,¹ hypoosmolality could result in enhanced activity of lipoprotein lipase, leading to an increase in glycerol release from circulating triglyceride-rich lipoproteins. However, this does not seem to explain the observed increase in the glycerol Ra during hypoosmolar conditions, since hypoosmolality had no significant effect on plasma triglyceride concentrations. It would have been expected that plasma triglyceride concentrations would decrease due to increased lipoprotein lipase activity and triglyceride hydrolysis.

The finding of a significantly higher glycerol Ra during hyperinsulinemic-euglycemic clamping strongly suggests that the antilipolytic action of insulin was partially overcome by hypoosmolar conditions in vivo. This is in contrast to previous in vitro findings that insulin and hypoosmolality both exert similar metabolic effects.^{5,6} All previous studies on the effects of anisoosmolality on cell metabolism have been performed using perfused livers or isolated hepatocytes.⁵ Although lipolysis occurs in all triglyceride-storing tissues, adipose tissue lipolysis reflects the majority of all detectable lipolytic activity in vivo. However, whether anisoosmolality affects whole-body lipolysis has not been investigated previously. In summary, the findings of this study indicate that hypoosmolality and the associated expansion of extracellular water result in an increased lipolytic rate in vivo. Since the decrease in osmolality was of a degree frequently observed in clinical conditions, the findings may indicate a role for osmolality in the regulation of lipid, glucose, and protein metabolism in pathophysiological conditions.

ACKNOWLEDGMENT

The technical assistance of S. Vosmeer and K. Dembinski is gratefully acknowledged.

Table 2. Indirect Calorimetry During the Second Kinetic Study

Parameter	Control Study		Hypoosmolality Study	
	Baseline	Clamp	Baseline	Clamp
VCO_2 (mL/min)	196 ± 5	210 ± 6	193 ± 8	$213 \pm 8^*$
VO_2 (mL/min)	238 ± 4	$242 \pm 7^*$	236 ± 9	236 ± 10
RQ	0.83 ± 0.01	$0.88 \pm 0.01^*$	0.82 ± 0.01	$0.91 \pm 0.02^*$
Fat utilization (%)	44 ± 5	$29 \pm 7^*$	48 ± 4	$18 \pm 6^*$
Carbohydrate utilization (%)	34 ± 6	$50 \pm 4^*$	31 ± 4	$60 \pm 6^*$

Abbreviations: VCO_2 , CO_2 production; VO_2 , O_2 consumption.* $P < .01$.

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