Enhanced Energy Expenditure, Glucose Utilization And Insulin Sensitivity In VAMP8 Null Mice

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Running Title: VAMP8 regulation of glucose metabolism

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Objectives: Previous studies have demonstrated that the VAMP8 protein plays a complex role in the control of granule secretion, transport vesicle trafficking, phagocytosis and endocytosis. The present study was aimed to investigate the role of VAMP8 in mediating GLUT4 trafficking and therefore insulin action in mice.

Research Design and Methods: Physiological parameters were measured using Oxymax indirect calorimetry system in 12 weeks old VAMP8 null mice. Dynamic analysis of glucose homeostasis was assessed using euglycemic-hyperinsulinemic (EU) clamp coupled with trace radioactively labeled 2-deoxyglucose. Insulin stimulated GLUT4 protein expressions on muscle cell surface were examined by immunofluorescence microscopy.

Results: VAMP8 null mice display reduced adiposity with increased energy expenditure despite normal food intake and reduced spontaneous locomotor activity. In parallel, the VAMP8 null mice also had fasting hypoglycemia (84±11 vs 115±4), enhanced glucose tolerance with increased insulin sensitivity due to both increased basal and insulin-stimulated glucose uptake in skeletal muscle (0.19±0.04 vs. 0.09±0.01 mmol/kg/min during basal, 0.6±0.04 vs. 0.31±0.06 mmol/kg/min during clamp in red-gastrocnemius muscle, p<0.05). Consistent with a role for VAMP8 in the endocytosis of the insulin responsive glucose transporter GLUT4, sarcolemma GLUT4 protein levels were increased in both the basal and insulin-stimulated states without any significant change in the total amount of GLUT4 protein, or related facilitative glucose transporters present in skeletal muscle, GLUT1, GLUT3 and GLUT11.

Conclusions: These data demonstrate that in the absence of VAMP8, the relative subcellular distribution of GLUT4 is altered resulting in increased sarcolemma levels that can account for increased glucose clearance and insulin sensitivity.
On the other, it has been recently reported that VAMP8 functions to inhibit phagocytosis in immature dendritic cells, to induce membrane ruffles and bacterial entry into non-phagocytic cells and for the efficient endocytosis of the plasma membrane GLUT4 protein in adipocytes (16; 30). Thus, VAMP8 appears to have several functions that may be dependent upon the particular cell type and/or trafficking cargo material.

To further investigate the role of VAMP8, we have taken advantage of the VAMP8 null mouse (19) to examine the integrative physiology of glucose metabolism and GLUT4 trafficking in skeletal muscle in vivo. These data demonstrate that VAMP8 plays multiple roles in the whole body regulation of metabolism, energy expenditure and insulin sensitivity. Consistent with a role of VAMP8 in mediating GLUT4 endocytosis, skeletal muscle sarcolemma levels of GLUT4 were elevated in the VAMP8 null mice directly accounting for the increase in both basal and insulin-stimulated glucose uptake.

**RESEARCH DESIGN AND METHODS**

**Animals** - The control and VAMP8 knockout mice were maintained in a mixed C57Bl/6J-129SvJ background (19) for over 11 generations and were housed in a temperature-controlled environment with a 12-h light/12-h dark cycle. Food intake and weights were determined daily, and all studies were performed on 12-month old male mice. All animal protocols were performed in accordance with Albert Einstein College of Medicine of Yeshiva University IACUC (Institutional Animal Care and Use Committee) approval.

**Indirect calorimetry** - Metabolic measurement was performed using an Oxymax indirect calorimetry system (Columbus Instruments, Columbus, OH) (31). Mice were individually housed in the chamber for 72 h with lights on from 0800 to 2000 in an ambient temperature of 22–24°C. Food was available ad libitum during the dark cycle (feeding phase 0800–0000) and removed during the light cycle (fasting phase 0800–2000). Respiratory Exchange Rate (RER) measurements were determined under Oxymax system settings as follows: air flow, 0.6 l/min; sample flow, 0.5 l/min; settling time, 6 min; and measuring time, 3 min. Six mice of each genotype were measured. The system was calibrated against a standard gas mixture to measure O₂ consumed (\( V_{O2}, \text{ml/kg per hr} \)) and CO₂ generated (\( V_{CO2}, \text{ml/kg/h} \)). Metabolic rate (\( V_{O2} \)), respiratory quotient (ratio of \( V_{CO2}/V_{O2} \)) and activity (counts) were evaluated over a 24 h period. Energy expenditure was calculated as the product of the calorific value of oxygen (3.815 + 1.232 × respiratory quotient) and the volume of O₂ consumed.

**In Vivo Assessment of Insulin Action and Glucose Metabolism** - Five days before the experiment, the mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and two indwelling catheter with prefilled heparin (1000 u/ml) and Polyvidone-Povidone (PVP; 0.5 g/ml; Sigma;) were introduced in the right internal jugular vein and left carotid artery. The catheters were externalized through an incision in the skin flap behind the head, and the mice were returned to individual cages after the surgery. The mice were fully recovered from the surgery before the in vivo experiments, as reflected by their reaching preoperative weight.

**Euglycemic-hyperinsulinemic clamp** - After a 12 h fast, euglycemic-hyperinsulinemic (EU) clamps were conducted in conscious mice as previously described (32; 33) with minor modification. The 2-h EU clamp was conducted with a continuous infusion of human insulin (4 m\( \mu \)g/kg/min) and a variable infusion of 25% glucose to maintain glucose at 110 mg/dl. To determine Insulin-stimulated whole body glucose metabolism, was estimated using continuous infusion of [3-\( ^3H \)]glucose (0.1 \( \mu \)Ci/min; PerkinElmer Life Sciences). To determine the rate of basal glucose turnover, [3-\( ^3H \)]glucose (0.05 \( \mu \)Ci/min) was infused for 2 h (basal period)
with 5 μCi bolus before starting the EU clamp, and a blood sample was taken at the end of this basal period. To assess insulin-stimulated tissue-specific glucose uptake, 2-deoxy-d-[1-14C][2-14C]DG glucose (PerkinElmer Life Sciences) was administered as a bolus (10 μCi) 75 min after the start of the clamp. Blood samples were taken at 0, and every 10 min from 80-120 min through carotid artery. To estimate basal muscle glucose uptake, 2-[^14C]DG glucose was infused with isotonic saline. During the clamp, plasma glucose was monitored using 2 μl of blood by glucose meter (precision Xtra, Abbot, Bedford, MA). Plasma [3-3H]glucose and 2-[14C] DG glucose, and 3H2O concentration were measured as described previously (32). After the EU-clamp, individual tissue samples were collected and immediately frozen at -80 °C for the measurement of glucose uptake.

**Oral glucose tolerance test (OGTT)** - Mice fasted for 12 h were administrated glucose (1g/kg) by oral. Plasma samples were collect at 0, 30, 60,120 min for glucose and insulin level. Glucose was measured using a glucose meter (precision Xtra, Abbot, Bedford, MA). Insulin levels were determined by enzyme-linked immunosorbent assay (ELISA) (Crystal Chem Inc. Downers Grove, IL). Membranes were immunoblotted with a VAMP8 polyclonal (18). Other antibodies were purchased from commercial suppliers: Glucose transporter 4 (GLUT4) polyclonal antibody (East Acres Biologicals Inc.), GLUT1, 3, 11 (Cell Signaling Technology, Inc.), UCP-1, UCP-2 (Abcam Inc.) and UCP-3 (Sigma). Quantification of all immunoblots was performed using NIH IMAGE software.

**Dynamic Glucose disposal** – The stable isotope labeled intraperitoneal glucose tolerance test (SipGTT) was performed as previously described (34). Briefly, male wild type and VAMP8 null mice were overnight fasted and were given a 1.0 g/kg intraperitoneal injection of glucose (containing 50% [6,6-2H2]-glucose). The amount of the M+2 label in the C3–C6 fragment for [6,6-2H2]glucose was determined by electron impact ionization mass spectrometry, after conversion of the glucose to a glucose aldonitrile pentaacetate derivative (35).

**Acute insulin stimulation** - For acute insulin stimulation, the mice were fasted for 12 h and given either isotonic saline or human recombinant insulin (1U/kg) by intraperitoneal injection. Fifteen min later the animals were anesthetized with pentobarbital sodium (50 mg/kg), and tissue samples were collected and subjected to immunblotting and immunofluorescence.

**Immunoblotting** - Tissues isolated from acute insulin stimulation were homogenized in ice-cold lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.25% sodium deoxycholate, 1 mM NaF, 1 mM Na3VO4, and 2 mM Na4P2O7) containing a protease inhibitor mixture (Roche Diagnostics). The resultant lysates were centrifuged at 16,000 × g for 60 min at 4 °C, and protein concentrations were quantified using the BCA (bicinchoninic acid) protein assays (Pierce). The protein samples (30 μg) were separated on a 4-12% gradient SDS-PAGE gel and transferred to nitrocellulose membranes using a semidy electromblotter (Owl Separation System, Portsmouth, NH). Membranes were immunoblotted with a VAMP8 polyclonal (18). Other antibodies were purchased from commercial suppliers: Glucose transporter 4 (GLUT4) polyclonal antibody (East Acres Biologicals Inc.), GLUT1, 3, 11 (Cell Signaling Technology, Inc.), UCP-1, UCP-2 (Abcam Inc.) and UCP-3 (Sigma). Quantification of all immunoblots was performed using NIH IMAGE software.
The slides were visualized by confocal fluorescent microscopy (model LSM510; Carl Zeiss MicroImaging, Inc.). Quantification of cell surface immunofluorescence was performed using NIH IMAGE software. 

**Statistical Analysis** - Results are presented as the means ± S.E.M. Statistical significance was determined using an unpaired two-tailed Student's *t* test, with *p* < 0.05 considered significant.

**RESULTS**

**VAMP8 null mice have reduced adiposity despite normal food intake.** To examine the metabolic phenotype of the VAMP8 null mice, we first determine total body weight, total water, lean and fat mass of male VAMP8 null mice compared to wild type controls by qNMR non-invasive imaging (Fig. 1). The VAMP8 null mice had reduced total body weight with no statistically significant difference in lean mass or total water content compared to control mice. In contrast, there was a marked reduction in total fat mass (Fig. 1A). This occurred despite normal caloric intake during both the light and dark cycle (Fig. 1B). These observed changes in fat mass was confirmed by tissue dissection that confirmed no significant difference in gastrocnemius muscle (Fig. 1C) or liver (Fig. 1D) weight but with a marked reduction in epididymal adipose tissue mass (Fig. 1E). The reduced adiposity resulted from a large reduction in adipocyte cell size as observed by histological imaging (Supplemental Fig. 1 in the online appendix available at [http://diabetes.diabetesjournals.org](http://diabetes.diabetesjournals.org)).

Since changes in adipocyte cell size have been correlated with changes in the secretion and plasma levels of various cytokines, we next determined the plasma levels of leptin, resistin, retinol binding protein 4 (RBP4) and adiponectin in the fasted state (Fig. 2). Consistent with reduced adipocyte cell size in the VAMP8 null mice, leptin and RBP4 levels were significantly decreased whereas adiponectin levels were somewhat increased. However, there was no significant difference in plasma resistin levels between the VAMP8 null and wild type control mice.

**VAMP8 null mice display increased energy expenditure and insulin sensitivity.** As the VAMP8 null mice have reduced adiposity despite equivalent daily caloric intake, we hypothesized that these mice would display increased energy expenditure. This speculation would be consistent with increased levels of adiponectin. To determine whole body energy expenditure, wild type and VAMP8 null mice were subjected to indirect calorimetry (Fig. 3). Oxygen consumption was increased both during the light and dark cycle resulting in a total 24 h increase corresponding to a 40% increase in total energy expenditure (Fig. 3A and B). This increase in energy expenditure also occurred even though the VAMP8 null mice also displayed a decrease in locomotor activity during the dark cycle (Fig. 3C).

As increased energy expenditure can result from mitochondrial uncoupling, we examined the expression of UCP-1/2/3 in skeletal muscle and adipose tissue (Supplemental Fig. 2). There was no significant difference in UCP-1 protein levels between wild type and VAMP8 null mice in brown adipose tissue, white adipose tissue or red gastrocnemius skeletal muscle, although we were unable to detect UCP-1 protein in white gastrocnemius skeletal muscle. Similarly, there was no change in UCP-3 levels in any of the tissues examined. In contrast, UCP-2 protein levels were increased in white gastrocnemius skeletal muscle of the VAMP8 null mice with no change in white adipose tissue or red gastrocnemius skeletal muscle.

In any case, the increase in energy expenditure and reduced adiposity resulted in marked enhancement of glucose tolerance along with a decrease in fasting plasma glucose levels (Fig. 4A). Although insulin levels were somewhat reduced in both the fasting state and during an oral glucose
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tolerance test (OGTT), the changes in insulin levels paralleled the changes in plasma glucose suggesting that total pancreatic beta cell insulin secretion was not significantly affected (Fig. 4B). Similarly, stable isotope labeled glucose ([6,6-^2H_2]-glucose) intraperitoneal tolerance test demonstrated a greater clearance of glucose in the VAMP8 null mice compared controls (Fig. 4C). Quantification of the area under the curve (AUC) showed that the VAMP8 null mice display a reduction in glucose excursion, increased glucose disposal with a concomitant reduction in total insulin secretion levels (Fig. 4D).

To specifically determine whether the VAMP8 null mice display increased insulin sensitivity we performed conscious non-stressed euglycemic-hyperinsulinemic (EU) clamps. As observed during the OGTT, in the basal or fasting level of plasma glucose was reduced in the VAMP8 null mice compared to wild type control and was normalized to the same level (~90 mg/dl during the EU-clamp (Fig. 5A). Similarly, plasma insulin was normalized to the same hyperinsulinemic levels during the EU-clamp for both the wild type and VAMP8 null mice (Fig. 5B). Consistent with an increase in insulin sensitivity, the glucose infusion rate during the EU-clamp was greater in the VAMP8 null along with increased whole body glucose uptake (Fig. 6A and B). The overall rate of body glycogen/lipid synthesis was also increased with no statistically significantly change in total body glycolysis rate, although there was a consistent increased trend in the VAMP8 null mice (Fig. 6C and D). Skeletal muscle accounts for the majority of glucose post-prandial glucose uptake and was significantly increased in red type 1 skeletal muscle fibers (soleus and red gastrocnemius) in both the basal and insulin-stimulated state (Fig. 7A). In parallel, insulin induced a greater suppression of hepatic glucose output in the VAMP8 null mice (Fig. 7B).

**VAMP8 null mice have increased sarcolemma levels of GLUT4 protein.**

Since the VAMP8 null displayed increased insulin-stimulated glucose uptake and fasting hypoglycemia, we speculated that the sarcolemma distribution of GLUT4 might be increased. To examine this possibility, red gastrocnemius muscle from overnight fasted wild type and VAMP8 null mice were fixed and subjected to confocal immunofluorescence for the endogenous GLUT4 protein (Fig. 8A). The muscle fiber from the VAMP8 null mice displayed an increase in the cell surface distribution of GLUT4 as co-localized with the sarcolemma marker protein a-dystroglycan. The increased muscle cell surface distribution of GLUT4 in the VAMP8 null mice was even more pronounced in the insulin-stimulated state (Fig. 8B). Quantification of these data demonstrated a statistically significant increase in the sarcolemma GLUT4 protein of the VAMP8 null mice in the basal and insulin-stimulated state (Fig. 8C). Similar to the gastrocnemius muscle, confocal immunofluorescence of soleus muscle also demonstrated increased basal and insulin-stimulated sarcolemma GLUT4 localization in the VAMP8 null mice compared to the control wild type mice (Supplemental Fig. 3). This change in GLUT4 subcellular distribution occurred with no significant change in the total expression levels of the GLUT4 protein or the total protein levels of several other facilitative glucose transporters GLUT1, GLUT3 and GLUT11 (Supplemental Fig. 4).

**DISCUSSION**

Loss of VAMP8 protein levels results in the impairment of multiple distinct membrane trafficking process including the terminal membrane fusion event of cytokinesis, zymogen granule release in pancreatic acinar cells, degranulation of mast cells, transcytosis in polarized epithelial cells, secretory granule release from platelet and cytotoxic T-cells and the plasma membrane exocytosis of the cystic fibrosis membrane conductance regulator and the aquaporin 2
water channel (22; 24; 26; 27; 29; 30; 37-42). In contrast, other studies have reported alternative roles for VAMP8 in membrane trafficking. For example, VAMP8 function is necessary for homotypic fusion of early and late endosomes, endosome-lysosome fusion, enhanced vesicle recycling between endocytic vesicles and the endosome in pancreatic beta cells and for GLUT4 endocytosis in adipocytes (15; 17; 21; 32; 43).

To examine the physiologic role of VAMP8 in glucose homeostasis in vivo, we took advantage of the VAMP8 null mice (19). Surprisingly, despite the alterations of various intracellular trafficking events, these mice displayed a marked reduction in adipocyte cell size with increased secretion levels of the adipokine adiponectin that favor enhanced glucose metabolism through increased fatty acid oxidation (44-47). More remarkably, the VAMP8 null mice have reduced spontaneous activity with identical caloric intake compared to the wild type mice, yet rather than gaining adipose tissue mass the VAMP8 null mice are leaner apparently due to increased total body energy expenditure. The increase in whole body energy expenditure was not due to changes in adipose tissue or skeletal muscle UCP-1 or UCP-3 expression. Although UCP-3 is the predominant isoform present in skeletal muscle with lower levels of UCP-2 (48; 49), we observed a significant increase in UCP-2 protein levels specifically in white gastrocnemius skeletal muscle of the VAMP2 null mice. Thus, it remains possible that the elevated UCP-2 levels account for the increase in energy expenditure and the reduced size of adipocytes could result from an increased demand of skeletal muscle for fatty acid oxidation. Future studies will be necessary to directly assess this possibility and the mechanism responsible for the tissue-specific UCP-2 induction.

The enhanced metabolic balance of the VAMP8 null mice was also reflected in increased whole body glucose tolerance and insulin sensitivity. Although there was a small enhancement in insulin suppression of hepatic glucose output the major effect was a marked increase in skeletal muscle glucose uptake in both the basal and the insulin-stimulated state. The increase in glucose uptake in the basal state is also consistent with the relative fasting hypoglycemia in the VAMP8 null compared to wild type mice. Mechanistically, the loss of VAMP8 resulted in a greater distribution of the skeletal muscle GLUT4 protein at the sarcolemma membrane in the basal state that is consistent with the increase in basal glucose uptake. Similarly, the greater extent of insulin-stimulated GLUT4 translocation to the sarcolemma is also consistent with the increase in insulin-stimulated glucose uptake. The increase in cell surface GLUT4 levels occurred without any change in total GLUT4 protein levels. Thus, the loss of VAMP8 function could either result in an increase in the rate of GLUT4 exocytosis and/or decrease in sarcolemma GLUT4 endocytosis. As we have previously observed that VAMP8 has no effect on GLUT4 exocytosis but is required for efficient GLUT4 endocytosis in 3T3L1 adipocytes (15), these data provide in vivo support for VAMP8 providing a necessary function in the cell surface endocytosis of the GLUT4 protein.

The glucose metabolic characteristics in the VAMP8 null mice is remarkably analogous to transgenic mice over expressing the GLUT4 gene both in normal GLUT4 target tissues as well as specifically in skeletal muscle (50-53). In all these models, over expression of GLUT4 resulted in increased cell surface levels of the GLUT4 protein that resulted in reduced fasting glycemia, increased skeletal muscle glycogen content, improve glucose tolerance and insulin sensitivity in peripheral tissue. This enhanced glucose handling previously reported for GLUT4 transgenic mice parallels the same reduction in fasting glucose, increased glycogen synthesis, increased insulin sensitivity and glucose tolerance observed in the VAMP8 null mice. Moreover, the reduction in insulin levels that follows the reduction in plasma glucose was accompanied by compensatory lipolysis that is also consistent with the reduction of adipocyte
cell size observed in the VAMP8 null mice.

Although all these metabolic data are consistent with VAMP8 playing a role in modulating skeletal muscle cell surface GLUT4 levels, how then do we also account for the observations that a variety of exocytotic secretory events are inhibited in VAMP8 null mice? One simple possibility is that VAMP8 plays different roles in the membrane trafficking of different cell types and/or for different cargo proteins. Alternatively, a more interesting possibility is the presence of a direct coupling between the endocytosis and exocytosis process. Previously it was reported that inhibition of chromaffin cell endocytosis by blocking dynamin function with a dynamin antibody resulted in a progressive decrease in catecholamine release (54). Similarly, blockade of dynamin function with a dominant-interfering mutant or reduction in dynamin protein levels by siRNA resulted in relatively normal initiation of first phase secretion but that was subsequently inhibited along with the more prolonged inhibition of second phase secretion in cultured beta cells (55). These data suggest the presence of a direct coupling between granule exocytosis (fusion pore opening) and endocytosis (fusion pore closure) events. Thus another possibility is that the extent of membrane exocytosis is directly coupled to endocytosis in a manner necessary to prevent unrestricted expansion of the plasma membrane surface area. In this case, blockade of VAMP8 function also results in an inhibition of membrane exocytosis via a feedback mechanism arising from the prevention of normal membrane endocytosis and recycling.

In either case, the data presented demonstrate that the loss of VAMP8 expression has a marked effect on the integrative physiology of glucose metabolism and energy expenditure. These combined effects results in a leaner mouse with increased energy expenditure and glucose disposal and insulin sensitivity. Thus, the development of a therapeutic strategy to inhibit VAMP8 function in skeletal muscle may be a viable approach to enhance glucose homeostasis.

**Author contributions.** H.Z. performed experiments, contributed to discussion and drafted manuscript. C-C.W. contributed to discussion, reviewed and edited manuscript. B.V. performed experiments, contributed to discussion, reviewed and edited manuscript. I.J.K contributed to discussion, reviewed and edited manuscript. W.H. contributed to discussion, reviewed and edited manuscript. J.E.P. contributed to discussion, reviewed, wrote and edited manuscript.

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**FIGURE LEGENDS**

**Figure 1.** VAMP8 null mice have a selective reduction in adipose tissue mass with normal food intake. A) Total adipose tissue, lean and water mass was determined in 12 weeks-old male wild type (WT, open boxes) and VAMP8 null mice (VAMP8, filled boxes) by quantitative NMR. B) The average caloric intake of a normal chow diet during both the light (7AM - 7PM) and dark (7PM – 7AM) cycle was determined in an Oxymax open-circuit indirect calorimetry system over a four-day period. C) Gastrocnemius skeletal muscle mass, D) liver mass and E) epididymal adipose tissue mass per total body weight was determined by dissection and weighing of the individual tissues. These data represent the mean ± standard error of mean (SEM) from 5 individual mice per group *p*<0.05.

**Figure 2.** VAMP8 null mice have changes in plasma levels of several adipokines. Fasting plasma levels of A) leptin, B) retinol binding protein 4 (RBP4), C) resistin and D) adiponectin in wild type (WT, open boxes) and VAMP8 null (VAMP8, filled boxes) mice were determined by ELISA analysis. These data represent the mean ± SEM from 6 mice per group. *p*<0.02, **p<0.002.

**Figure 3.** Loss of VAMP8 expression results in increased energy expenditure (EE). Fourteen weeks old wild type (WT, open boxes) and VAMP8 null (VAMP8, filled boxes) mice were placed into Oxy max open-circuit indirect calorimetry system over a four-day period. The mice were fed in the dark (7PM - 7AM) cycle and food restricted during the light cycle (7AM – 7PM). The average oxygen consumption in ml/kg/h (A) and energy expenditure in kcal/gm/h (B) were determined. C) Spontaneous locomotor activity in the x, y and z planes were determined as the total number of beam brakes. These are the averages from 5 wild type and 4 VAMP8 null mice. *p*<0.05, **p<0.003.

**Figure 4.** VAMP8 null mice display enhanced glucose tolerance. Twelve weeks old wild type (WT, open circles) and VAMP8 null (VAMP8, filled circles) were fasted for 14 h and given 1 g/kg of D-glucose in isotonic saline by oral gavage. Blood (10 ul) was collected from the tail vein at 0, 30, 60 and 120 min. The blood glucose (A) and insulin (B) levels were determined and represent the mean ± SEM from 5 individual mice per group. *p*<0.05, **p<0.003. C) Twelve weeks old wild type and VAMP8 null were fasted for 15 hours and given an intraperitoneal injection of 1g/kg of D-glucose 50% enriched with [6,6-2H2]-glucose. Each point represents the mean ± SEM from 4 individual mice per group. * p<0.05, **p<0.001. D) The area under the curve (AUC) for blood glucose and insulin levels were calculated from the glucose and glucose isotopomers measured during the stable isotope glucose tolerance tests.
Figure 5. Determination of plasma glucose and insulin levels during euglycemic-hyperinsulinemic (EU) clamps. A) Plasma glucose and (B) plasma insulin level during basal and last 30 min of the EU-clamps (4 mU/kg/min insulin infusion with glucose level maintained at ~90 mg/dl) for the wild type (WT, open boxes) and VAMP8 null (VAMP8, filled boxes) mice. These data represent the mean ± SEM from 7-10 individual mice per group. *p<0.05.

Figure 6. VAMP8 null mice have increased whole body insulin sensitivity, glucose uptake and glycogen/lipid synthesis. Wild type (WT, open boxes) and VAMP8 null (VAMP8, filled boxes) were subjected to hyperinsulinemic-euglycemic (EU) clamps to determine (A) the glucose infusion rate (GIR) necessary to maintain euglycemia. B) Whole body glucose uptake was determined as [3-3H]-glucose specific activity tracer infusion rate and weight of mice in the basal or EU-clamp state. C) The whole body glycolysis was determined from the increment of the plasma 3H2O concentration multiplied by the estimated body water divided by [3-3H]-glucose specific activity. (D) The glycogen/lipid synthesis was estimated as the difference between whole body glucose uptake and whole body glycolysis. These data represent the mean ± SEM from 7-10 individual mice per group. * p<0.05.

Figure 7. VAMP8 null mice have increased skeletal muscle glucose uptake with decreased hepatic glucose output. A) Basal and insulin-stimulated glucose uptake into skeletal muscle tissues (soleus and red gastrocnemius (GAS-R) were determined by 2-deoxy-D-[1-14C] glucose injection during the last 35 min of saline (Basal) or insulin infusion during the EU-Clamp. B) The insulin suppression of hepatic glucose output was determined during the EU-clamp by [3-3H]-glucose infusion. These data represent the mean ± SEM from 5-10 individual mice per group. * p<0.05.

Figure 8. VAMP8 null mice have increased GLUT4 protein localized to the sarcolemma membrane in red gastrocnemius skeletal muscle. Wild type (WT) and VAMP8 null (VAMP8) mice were fasted overnight (12 h) or given an intraperitoneal injection of either (A) isotonic saline or (B) insulin (1 U/kg). Fifteen min later the red gastrocnemius (Gas-R) muscle was isolated, and embedded in OCT as described in Research Design and Methods. The frozen tissue cross-sections (10 μm) were then subjected to confocal immunofluorescence with specific GLUT4 and the α-dystroglycan (αDG) antibody. Representative images are shown and quantification of the relative extent of cell surface distribution of GLUT4 (C) was determined as the mean ± SEM from 5-10 independent mice. *p<0.05, **p<0.003, WT mice (open boxes) and VAMP8 mice (filled boxes).
Figure 1

- Body Composition
- Food Intake
- Muscle Weight
- Liver Weight
- VAT Weight

Figure 2

- Leptin
- RBP4
- Resistin
- Adiponectin

Figure 3

- FFA
- KI
- GLUT4
Figure 4

A) Glucose (mg/dL) over time.

B) Insulin (µg/L) over time.

C) [6,6-2H]glucose (mg/dL) over time.

D) AUC (X 120 min).

Figure 5

A) Plasma Glucose (mg/dL).

B) Plasma Insulin (µg/L).

Figure 6

A) Glucose (mg/dL).

B) Glucose uptake (µg/kay).

C) Glucose synthesis (µg/kay).

D) Synthesis (µg/kay).
Figure 7

![Graph A and B]

Figure 8

![Images of GLUT4 and Merge with Bar Graph C]