mRNA expression of diacylglycerol kinase isoforms in insulin-sensitive tissues: effects of obesity and insulin resistance

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Abstract
Diacylglycerol kinase (DGK) isoforms regulate signal transduction and lipid metabolism. DGKδ deficiency leads to hyperglycemia, peripheral insulin resistance, and metabolic inflexibility. Thus, dysregulation of other DGK isoforms may play a role in metabolic dysfunction. We investigated DGK isoform mRNA expression in extensor digitorum longus (EDL) and soleus muscle, liver as well as subcutaneous and epididymal adipose tissue in C57BL/6J mice and obese and insulin-resistant ob/ob mice. All DGK isoforms, except for DGKζ, were detectable, although with varying mRNA expression. Liver DGK expression was generally lowest, with several isoforms undetectable. In soleus muscle, subcutaneous and epididymal adipose tissue, DGKδ was the most abundant isoform. In EDL muscle, DGKα and DGKζ were the most abundant isoforms. In liver, DGKζ was the most abundant isoform. Comparing obese insulin-resistant ob/ob mice to lean C57BL/6J mice, DGKβ, DGKι, and DGKθ were increased and DGKε expression was decreased in EDL muscle, while DGKβ, DGKη and DGKθ were decreased and DGKδ and DGKζ were increased in soleus muscle. In liver, DGKδ and DGKζ expression was increased in ob/ob mice. DGKζ was increased in subcutaneous fat, while DGKζ was increased and DGKβ, DGKδ, DGKη and DGKε were decreased in epididymal fat from ob/ob mice. In both adipose tissue depots, DGKα and DGKγ were decreased and DGKζ was increased in ob/ob mice. In conclusion, DGK mRNA expression is altered in an isoform- and tissue-dependent manner in obese insulin-resistant ob/ob mice. DGK isoforms likely have divergent functional roles in distinct tissues, which may contribute to metabolic dysfunction.

Introduction
Increased accumulation of lipid intermediates, such as triglycerides, diacylglycerol (DAG), ceramides, and long-chain fatty acid coenzyme A, contribute to the development of insulin resistance (Erion and Shulman 2010; Samuel and Shulman 2012; Zhang et al. 2013). Ectopic accumulation of specific lipid metabolites (diacylglycerols and/or ceramides) in liver and skeletal muscle may be caused by increased fatty acid delivery/synthesis when the adipose tissue storage capacity is exceeded and/or decreased mitochondrial fatty acid oxidation (Erion and Shulman 2010; Zhang et al. 2013). Increased intramuscular DAG activates a serine/threonine kinase signaling
pathway, including protein kinase C (PKC), which results in serine phosphorylation of insulin receptor substrate (IRS) -1, inhibition of IRS-1 tyrosine phosphorylation, and reduced insulin-stimulated glucose uptake and metabolism (Samuel and Shulman 2012). In liver, increased DAG content reduces insulin-stimulated glycogen synthesis and decreases the suppression gluconeogenesis (Samuel and Shulman 2012). Thus, altered activity and/or abundance of enzymes involved in lipid metabolism influences the accumulation of different lipid intermediates and impinges upon intracellular signaling events.

Diacylglycerol kinases (DGKs) control the level of two important lipid messengers: DAG and phosphatidic acid (PA). DGKs catalyze a reaction that removes DAG, by converting this lipid to PA at the plasma membrane, endoplasmic reticulum and nucleus, and thereby terminates DAG-derived signals (Shulga et al. 2011). Consequently, DGK isoform expression may be altered in obesity and type 2 diabetes. Ten mammalian DGK isoforms have been classified into five subgroups based on different regulatory domains in their primary structure, although all isoforms have a catalytic domain and at least two C1 domains (Shulga et al. 2011; Sakai and Sakane 2012). DGK isoforms are unique, not only structurally, but also in their expression pattern, subcellular localization, regulatory mechanisms and DAG preferences, suggesting isoform-specific functional roles. While DGK isoform expression has been surveyed in various tissues including in mouse and rat reproductive organs (Toya et al. 2005; Shionoya et al. 2015), immune cells during an inflammatory reaction (Yamamoto et al. 2014), rat retina (Hozumi et al. 2013), lung (Katagiri et al. 2005), and regenerating liver (Nakano et al. 2012), and human failing hearts (Bilim et al. 2011), mRNA expression of DGK isoforms in tissues important for metabolic homeostasis in the context of type 2 diabetes and obesity is unknown.

DGK isoforms regulating wide variety of physiological processes including growth, metabolism, proliferation, immunological and neural development (Topham 2006; Krishna and Zhong 2013; Ishisaka and Hara 2014; Shirai and Saito 2014; Yamamoto et al. 2014). DGKδ expression is decreased in skeletal muscle from type 2 diabetic patients and haploinsufficiency (DGKδ+/−) in mice leads to insulin resistance and late-onset obesity (Chibalin et al. 2008). However, the role of other DGK isoforms in the development of metabolic disorders, including insulin resistance and obesity, is largely unknown. Here, we determined the tissue-specific mRNA expression of DGK isoforms in insulin-sensitive tissues including skeletal muscle, liver and two separate adipose tissue depots (subcutaneous and epididymal). We also explore the effect of severe obesity and insulin resistance on DGK isoform mRNA expression by comparing tissue-specific profiles between obese ob/ob (B6.V-Lepob/J) and lean C57BL/6J mice.

Material and Methods

Animals

Male lean C57BL/6J and obese ob/ob (B6.V-Lepob/J) mice were purchased from Charles River Laboratories (Italy). Animals were maintained in a temperature- and light-controlled environment (12-h light, 12-h dark cycle) and had free access to water and food. At 12 weeks of age, fed mice were anesthetized via intraperitoneal injection of 2.5% Avertin (0.02 mL/g body weight). Skeletal muscles (extensor digitorum longus (EDL) and soleus), adipose tissues (subcutaneous and epididymal) and liver were harvested from lean C57BL/6J (body weight 24.4 ± 0.2 g) and obese ob/ob (body weight 47.7 ± 1.2 g) and clamp-frozen in liquid nitrogen and stored for mRNA analysis. The regional animal ethics committee of Northern Stockholm approved all experimental procedures.

RNA isolation, cDNA synthesis and qPCR

Tissues were homogenized using the TissueLyser (Qiagen, Hilden, Germany). Total RNA in adipose tissue and liver was isolated using RNeasy Lipid Tissue Mini Kit and RNeasy Mini Kit (Qiagen), respectively. A deoxyribonuclease I (QIAGEN) digestion step was included to eliminate DNA contamination. Total RNA in skeletal muscle was isolated using TRIzol reagent (Invitrogen Life Technologies Ltd, Paisley, UK), according to the manufacturer’s protocol. RNA concentration and purity were determined with a Nanodrop ND-1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA) and all samples had a 260/280 and a 260/230 ratio >2.0 indicating pure RNA. RNA integrity was verified with the Experion (Bio-Rad Laboratories, Hercules, CA) and all samples had a RQI value >8 indicating intact RNA. cDNA was synthesized from 1 or 2 μg total RNA in a final reaction volume of 20 μL using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Stockholm, Sweden), according to the manufacturer’s protocol.

Real-time qPCR

Real-time qPCR was performed in a StepOnePlus Real-time qPCR System (Applied Biosystems) using Taqman Gene Expression Assays (Table 1). Multiplex qPCR was performed in duplicate using 20 ng cDNA mixed with TaqMan Gene Expression Master Mix (Applied Biosystems) in a total volume of 20 μL. Cycling conditions were
Results are presented as mean ± SEM. Differences in gene expression between C57BL/6J and ob/ob mice were determined by Student’s t-test on logarithmic transformed data. Significance was accepted at \( P < 0.05 \).

### Results

#### Tissue expression profile in C57BL/6J mice

None of the reference genes were stably expressed across all investigated tissues (Table 2). Therefore, data are presented as \( C_q \) values (Table 3) and normalized against gene expression of each specific DGK isoform in EDL muscle (Fig. 1) for the comparison between isoforms in different tissues of C57BL/6J mice.

The DGKz gene was highly expressed in EDL muscle, but not expressed in soleus muscle and lowly expressed in liver and adipose tissue (Fig. 1A). DGKβ and DGKγ were not expressed in liver, while levels were similar between skeletal muscle and adipose tissues (Fig. 1B and C). DGKδ and DGKη were expressed at higher levels in adipose tissues compared to skeletal muscle, with very low levels in liver (Fig. 1D and E). A similar expression pattern was observed DGKζ (Fig. 1F). The expression profile for DGKζ and DGKθ between tissues was similar (Fig. 1G and I); however a threefold and sixfold higher expression, respectively, was observed in subcutaneous adipose tissue, and similar levels were observed between skeletal muscle, liver and epididymal adipose tissue. Overall, mRNA expression of DGKζ was higher than DGKθ (Table 2). DGKζ was 4-fold higher in soleus and subcutaneous adipose tissue compared to EDL and epididymal adipose tissue, while not detected in liver (Fig. 1H).

We next compared the relative isoform expression within each tissue. In EDL muscle, DGKz and DGKζ had the highest expression level (Table 3). While DGKz was not expressed in soleus muscle, DGKδ was the highest isoform expressed (Table 3 and Fig. 1). In general the expression level for all DGK isoforms was low in liver compared to other tissues, with DGKζ, DGKγ, DGKζ and DGKδ were under the detection limit of the assay (Table 3 and Fig. 1). The expression level of the DGK isoforms also differed between the two adipose tissue depots analyzed; DGKβ, DGKζ, DGKδ and DGKη were more highly expressed in epididymal adipose tissue as compared to subcutaneous adipose tissue with the inverse noted for DGKζ, DGKζ, DGKδ and DGKθ (Table 3 and Fig. 1). DGKδ had the highest expression in both adipose depots (Table 3). DGKζ was the only isoform with a \( C_q < 30 \) in all investigated tissues (Table 3). DGKβ, DGKζ, DGKη, DGKζ and DGKθ were expressed at low levels in all tissues (\( C_q > 30 \); Table 3) and DGKζ was not detected in any of the investigated tissues.

### Table 1. Taqman gene expression assays.

| Symbol | Name | Taqman Assay ID |
|--------|------|----------------|
| Dgka   | Diacylglycerol kinase, alpha (\( \alpha \)) | Mm00440488_m1* |
| Dgkb   | Diacylglycerol kinase, beta (\( \beta \)) | Mm00618478_m1* |
| Dgkg   | Diacylglycerol kinase, gamma (\( \gamma \)) | Mm00446756_m1* |
| Dgkd   | Diacylglycerol kinase, delta (\( \delta \)) | Mm00617404_m1* |
| Dgkh   | Diacylglycerol kinase, eta (\( \eta \)) | Mm01312241_m1* |
| Dgkk   | Diacylglycerol kinase, kappa (\( \kappa \)) | Mm01340751_m1* |
| Dgke   | Diacylglycerol kinase, epsilon (\( \epsilon \)) | Mm00444676_m1* |
| Dgkz   | Diacylglycerol kinase, zeta (\( \zeta \)) | Mm00661896_m1* |
| Dgki   | Diacylglycerol kinase, iota (\( \iota \)) | Mm01159464_m1* |
| Dgqk   | Diacylglycerol kinase, theta (\( \theta \)) | Mm01198794_m1* |

*The TaqMan gene expression assay with the best coverage for that specific gene.

as follows: 95°C for 10 min, 40 cycles at 95°C for 15 sec and 60°C for 1 min.

Data were analyzed with Step One Software v2.1 (Applied Biosystems). Relative gene expression was calculated with the \( \Delta \Delta C_q \) method (Livak and Schmittgen 2001) and expressed as fold changes compared with DGKz mRNA in C57BL/6J mice. Ten candidate reference genes (Table 1) were validated with NormFinder algorithm incorporated into the GenEx software (MultiD Analyses AB, Gothenburg, Sweden). The relative expression was normalized in EDL against the reference genes Gusb, Hprt1 and Ppia, in soleus against the reference genes 18S, Actb, Hprt1, Ppia, Rplp0 and Tbp, in liver against the reference genes Actb, B2 m, and Ppia, in subcutaneous adipose tissue against the reference genes B2 m, Pgtk, Ppia, and Tbp, and in epididymal adipose tissue against the reference genes Pgtk and Ppia. Isoforms with a \( C_q > 35 \) were considered below the limit of detection.

### Statistics

Results are presented as mean ± SEM. Differences in gene expression between C57BL/6J and ob/ob mice were
Several DGK isoforms were differentially expressed between ob/ob and C57BL/6J mice. In EDL muscle, DGKζ was the predominant isoform, followed by DGKα, DGKδ and DGKε (Fig. 2A). Moreover, expression of DGKβ, DGKδ and DGKθ was increased, whereas DGKε was decreased in EDL muscle from ob/ob compared to C57BL/6J mice (Fig. 2A). In soleus muscle, DGKδ was the predominant isoform followed by DGKζ and DGKε (Fig. 2B). Expression of DGKδ and DGKε was increased, whereas DGKβ, DGKη, and DGKθ were decreased in soleus of ob/ob compared to C57BL/6J mice (Fig. 2B). In liver, DGKζ was the predominant isoform followed by DGKz (Fig. 3). Liver DGKδ and DGKζ expression was increased in ob/ob compared to C57BL/6J mice (Fig. 3). In subcutaneous adipose tissue, DGKδ was the predominant isoform followed by DGKζ (Fig. 4A). The DGKη and DGKε expressions were increased and the DGKz and DGKγ expressions were decreased in subcutaneous adipose tissue of ob/ob compared to C57BL/6J mice (Fig. 4A). In epididymal adipose tissue, DGKδ was the predominant isoform followed by DGKζ (Fig. 4B). DGKζ and DGKε expression was increased and DGKz, DGKβ, DGKδ, DGKθ, DGKζ, and DGKε expression was decreased in epididymal adipose tissue of ob/ob compared to C57BL/6J mice (Fig. 4B).

**Table 2.** mRNA expression of reference genes in insulin-sensitive tissues from C57BL/6J mice.

| Gene   | EDL          | Soleus       | Liver         | Subcutaneous | Epididymal |
|--------|--------------|--------------|---------------|--------------|------------|
| Rn18S  | NA           | 9.66 ± 0.06  | NA            | 8.83 ± 0.12  | NA         |
| Actb   | NA           | 23.84 ± 0.07 | 21.95 ± 0.10  | 18.18 ± 0.23 | NA         |
| B2m    | 24.65 ± 0.12 | 24.61 ± 0.08 | 20.25 ± 0.08  | 19.86 ± 0.14 | NA         |
| Gapdh  | NA           | 19.79 ± 0.05 | NA            | 20.56 ± 0.15 | 19.47 ± 0.14|
| Gusb   | 29.36 ± 0.12 | 29.17 ± 0.09 | NA            | 26.49 ± 0.12 | NA         |
| Hprt1  | 27.58 ± 0.07 | 27.86 ± 0.07 | NA            | 25.45 ± 0.12 | 24.93 ± 0.10|
| Pgk1   | 22.88 ± 0.09 | 25.14 ± 0.08 | 25.09 ± 0.07  | 25.51 ± 0.11 | 26.20 ± 0.11|
| Ppia   | 24.93 ± 0.08 | 25.61 ± 0.06 | 24.44 ± 0.09  | 21.93 ± 0.12 | 20.92 ± 0.11|
| Rplp0  | 22.35 ± 0.06 | 21.98 ± 0.10 | NA            | 20.25 ± 0.20 | NA         |
| Tbp    | NA           | 31.56 ± 0.10 | NA            | 30.85 ± 0.15 | NA         |

Data are presented as Cq values and expressed as mean ± SEM. NA, not applicable.

**Table 3.** DGK isoform mRNA expression in insulin-sensitive tissues from C57BL/6J mice.

| Gene   | EDL          | Soleus       | Liver         | Subcutaneous | Epididymal |
|--------|--------------|--------------|---------------|--------------|------------|
| DGKα   | 27.6 ± 0.1   | ND           | 30.8 ± 0.1    | 32.3 ± 0.5   | 33.8 ± 0.3 |
| DGKβ   | 32.0 ± 0.1   | 32.7 ± 0.2   | ND            | 33.8 ± 0.5   | 31.9 ± 0.2 |
| DGKδ   | 33.2 ± 0.1   | 32.4 ± 0.1   | ND            | 33.2 ± 0.2   | 31.9 ± 0.3 |
| DGKζ   | 29.2 ± 0.1   | 26.8 ± 0.1   | 34.4 ± 0.4    | 25.4 ± 0.2   | 24.8 ± 0.1 |
| DGKε   | 31.8 ± 0.1   | 31.6 ± 0.1   | 33.7 ± 0.2    | 30.6 ± 0.1   | 29.9 ± 0.3 |
| DGKz   | ND           | ND           | ND            | ND           | ND         |
| DGKθ   | 29.7 ± 0.1   | 28.9 ± 0.1   | 33.0 ± 0.1    | 28.5 ± 0.1   | 28.6 ± 0.1 |
| DGKη   | 27.4 ± 0.1   | 27.8 ± 0.1   | 28.5 ± 0.1    | 25.9 ± 0.3   | 28.1 ± 0.1 |
| DGKκ   | 34.7 ± 0.2   | 32.9 ± 0.1   | ND            | 32.7 ± 0.2   | 34.0 ± 0.6 |
| DGKθ   | 33.2 ± 0.1   | 32.8 ± 0.1   | 33.5 ± 0.2    | 30.6 ± 0.1   | 32.7 ± 0.1 |

Data are presented as Cq values and expressed as mean ± SEM. ND, not detected.
Figure 1. DGK isoform expression profile. mRNA expression of (A) DGKα, (B) DGKβ, (C) DGKγ, (D) DGKδ, (E) DGKη, (F) DGKε, (G) DGKζ, (H) DGKι and (I) DGKθ in EDL muscle, soleus muscle, liver, subcutaneous adipose tissue, and epididymal adipose tissue from C57BL/6J mice. Data are presented relative to respective mRNA in EDL muscle. Results are mean ± SEM. n = 8–10.
the predominant isoform in subcutaneous and epididymal adipose tissue, consistent with earlier reports (Lowe et al. 2013; Shulga et al. 2013). Likewise, DGKδ was highly expressed in skeletal muscle, consistent with human RNA-seq expression data (Krupp et al. 2012). Isoforms in the same subfamily did not necessarily share same expression profile and isoforms from different subfamilies were expressed at similar magnitude, further highlighting the tissue-specific roles of DGK isoforms.

Several genetically modified DGK mouse models have been generated (Rodriguez de Turco et al. 2001; Zhong et al. 2003; Regier et al. 2005; Crotty et al. 2006; Olenchock et al. 2006; Chibalin et al. 2008; Shirai et al. 2010), each demonstrating distinct phenotypic traits, further implying functional diversity in DGK isoform-specific signaling (Shirai and Saito 2014). Given that both the substrate (DAG) and the product (PA) of the reaction catalyzed by DGK serve as metabolic intermediates and potent signaling molecules, an imbalance in these substrates may have an impact on cell growth and metabolism. Ectopic intracellular accumulation of DAG is associated with insulin resistance in skeletal muscle and liver (Erion and Shulman 2010; Samuel and Shulman 2012; Zhang et al. 2013; Zachariah Tom et al. 2014). Consequently, an imbalance in expression of DGKs may have a role in the pathogenesis of various metabolic disorders including type 2 diabetes, obesity, and nonalcoholic fatty liver disease. Thus, we next...
determined the expression of DGK isoforms in obese insulin-resistant ob/ob mice.

DGKα, DGKβ and DGKγ belong to the type I subgroup of DGKs, which have a calcium-binding EF hand motif (Shulga et al. 2011). DGKα is abundant in T cells and has a role in T-cell activation and T-cell anergy (Olenchock et al. 2006). In healthy humans, DGKα mRNA levels are comparable between adipose tissue, liver and skeletal muscle, DGKγ mRNA is increased in skeletal muscle compared to liver and adipose tissue, whereas DGKβ is minimally expressed in these tissues (Krupp et al. 2012). DGKα and DGKγ are highly expressed in mouse pancreatic β-cells and diminished levels of these isoforms attenuate insulin secretion (Kurohane Kaneko et al. 2013), implying a role in metabolic disease. Indeed, mRNA of these isoforms was reduced in subcutaneous and epididymal adipose tissue of ob/ob mice, but the functional significance is unknown. Genetic studies have linked DGKβ to diabetes risk. A meta-analysis of 21 genome-wide association studies found an association between the loci containing DGKβ (DGKB-TMEM195) and type 2 diabetes (Dupuis et al. 2010). Even though this isoform was lowly expressed, DGKβ mRNA was increased EDL muscle, and concomitantly decreased in soleus muscle and epididymal adipose tissue from ob/ob mice. Although DGKβ may be involved in type 2 diabetes, knockout mice show attention-deficit behavior and a hyperactive phenotype, implicating a predominant role in neurological, rather than metabolic disorders (Ishisaka et al. 2012).

The members of type II DGKs, DGKδ, DGKζ, and DGKκ, have pleckstrin homology domains and sterile α motifs (Sakai and Sakane 2012). We found that DGKδ and DGKζ mRNA was readily detected in all insulin-sensitive tissues, whereas DGKκ was undetected. RNA-Seq data show DGKκ is lowly expressed in most tissues, with the highest expression noted in testes and brain (Krupp et al. 2012). In contrast, DGKδ is widely expressed in insulin-sensitive tissue (Chibalin et al. 2008; Krupp et al. 2012) and plays a major role in regulating the synthesis of a broad range of lipid species (Shulga et al. 2013). DGKδ also increases lipid synthesis by promoting de novo fatty acid synthesis (Shulga et al. 2013). DGKδ+/− mice have increased DAG content in skeletal muscle, leading to impaired peripheral insulin sensitivity and age-dependent obesity (Chibalin et al. 2008). Here we report DGKδ is highly expressed in skeletal muscle and adipose tissue, and to a lower extent in liver, consistent with a previous study (Lowe et al. 2013). Total DGK activity is reduced in skeletal muscle and adipose tissue, but not liver from DGKδ+/− mice (Chibalin et al. 2008), indicating DGKδ plays a major role in peripheral tissues and a minor role in liver. In ob/ob mice, DGKδ mRNA was increased in liver and soleus muscle, decreased in epididymal adipose tissue and unaltered in subcutaneous adipose; further highlighting the impact of metabolic disease on DGKδ expression profiles. Hyperglycemia, a cardinal feature of type 2 diabetes, regulates DGKδ abundance and subcellular localization (Miele et al. 2007; Chibalin et al. 2008; Takeuchi et al. 2012), as well as DGKδ enzyme activity against palmitic acid-containing DAG species (Sakai et al. 2014). Interestingly saturated fatty acids increase and monounsaturated fatty acids attenuated
DGKδ mRNA expression in C2C12 cells (Sakiyama et al. 2014). Consequently, the level of glycemia or different lipid species in type 2 diabetes or obesity may impact the abundance and activity of DGKδ. DGKη regulates a wide variety of physiological events including cell growth and proliferation. DGKη is linked to bipolar disorder (Moya et al. 2010), lung cancer (Nakano et al. 2014), and heart failure (Bilim et al. 2011), but its functional role in metabolic disorder is unclear. In ob/ob mice, DGKη was slightly reduced in soleus muscle and epididymal adipose tissue, while increased in subcutaneous adipose tissue. While DGKδ plays a role in metabolic disease, DGKη regulates other fundamental processes including proliferation and differentiation via Ras/B-Raf/C-Raf/MEK/ERK signaling (Yasuda et al. 2009). Dissecting the role of DGKδ and DGKη along diverse metabolic and growth-promoting pathways may further elucidate the diverse pathogenesis of chronic disorders including insulin resistance, heart failure, sarcopenia, and cancer.

DGKζ is the only member of the type III DGKs and is distinguished by a unique structure and substrate specificity toward arachidonate-containing DAG (Shulga et al. 2011). DGKζ knockout mice have a higher resistance to seizures induced by electroconvulsive shock (Rodriguez de Turco et al. 2001), while transgenic mice overexpressing DGKζ are protected from experimental cardiac hypertrophy (Niizeki et al. 2008). DGKζ is expressed in various immune cells, with increased levels observed in response to inflammatory stimuli (Yamamoto et al. 2014). In humans, DGKζ is highly expressed in brain and spleen, with lower, but comparable levels in liver, skeletal muscle and adipose tissue (Krupp et al. 2012). We observed DGKζ expression was marginally reduced in EDL muscle and appreciably reduced in epididymal adipose tissue from ob/ob mice. This was unexpected given that overexpression of DGKζ in muscle cells leads to defects in insulin signaling (Cazzoli et al. 2007).

The subfamily of type IV isoforms includes DGKε and DGKρ, which contain ankyrin repeats, a C-terminal nuclear localization signal and a PDZ-binding motif, as well as MARCKS homology region (Shulga et al. 2011). Analogous to DGKζ, DGKε plays a critical role in immune cells (Zhong et al. 2003). However, DGKε is ubiquitously expressed in most tissues and most predominant in the brain (Krupp et al. 2012), suggesting a role in several organ systems. We found DGKε to be highly expressed in all insulin-sensitive tissues. DGKε expression was increased in epididymal adipose tissue and liver from ob/ob mice, while similar levels were noted in subcutaneous adipose tissue, soleus muscle and EDL muscle compared to C57BL/6J mice. The second member in the type IV DGK group, DGKρ, is predominately expressed in brain, but lowly expressed in insulin sensitivity tissues (Krupp et al. 2012). We found DGKρ mRNA expression was increased in soleus and EDL muscle, as well as subcutaneous and epididymal adipose tissue from ob/ob mice. The expression of DGKρ decreases in 3T3-L1 cells during adipocyte differentiation (Shulga et al. 2013). Interestingly, the National Heart, Lung, and Blood Institute Family Heart Study (FHS) genome-wide linkage scan identified DGKρ as a candidate gene for influencing BMI (Laramie et al. 2009), but secondary validation and functional studies are required to confirm this association.

DGKθ, the single member of the type V DGK, has three C1 domains, a Gly/Pro-rich domain and a pleckstrin homology domain (Shulga et al. 2011). Overexpression of DGKθ in mouse hepatocytes increases PA and decreases DAG content, while concomitantly impairing insulin signaling (Zhang et al. 2014). Consistent with other DGK isoforms, DGKθ is highly expressed in different regions of the brain in rats (Houssa et al. 1997). We observed that DGKθ mRNA was increased in EDL muscle, decreased in soleus muscle and unaltered in liver and adipose tissue depots in ob/ob versus lean mice. Nevertheless, DGKθ mRNA was lowly expressed compared to other isoforms, similar to RNA-Seq data from human tissues (Krupp et al. 2012). DGKθ has emerged as a relevant target for metabolic regulation given evidence that this isoform acts as a key mediator of bile-acid-stimulated modulation PA-dependent mTOR and Akt signaling and glucose homoeostasis in HepG2 cells and primary human hepatocytes (Cai and Sewer 2013).

The identification of reference genes that are consistently expressed across several tissues is a frequent limitation, given the fact that even references genes often have a variable tissue-specific mRNA expression profile. To deal with this limitation, we profiled several reference genes expressed in the various metabolic tissues under study. Based on the results of this analysis, we were unable to identify a reference gene that was stable in all the tissues studied. Therefore, we present the Cq values and have normalized the data for the analysis comparing the expression of different DGK isoforms across the various tissues against the expression level in EDL muscle. Because we were unable to the normalize mRNA expression of the individual DGK isoforms between the various tissues to a common set of reference genes, we cannot exclude that this approach might have induced potential artifacts. When comparing mRNA expression profile of DGK isoforms within each tissue of C57BL/6J and ob/ob mice, the isoforms were normalized to the reference genes relevant for each tissue and then to a calibrator gene.

In summary, we provide evidence for tissue-specific expression profiles of DGK isoforms insulin-sensitive tissue from lean C57BL/6J mice. DGKδ is the most abundant isoform in soleus muscle, subcutaneous and
epididymal adipose tissue. DGKα and DGKζ are the predominant isoforms in EDL muscle. Finally, in liver, DGKζ is the predominant isoform, with comparable levels of DGKβ and DGKδ noted. Overall, mRNA expression of DGK isoforms was generally lower in liver compared to skeletal muscle and adipose tissue. In conclusion, DGK expression was altered in an isoform- and tissue-specific manner in obese insulin-resistant mice, suggesting DGKs play unique roles in each tissue and may play a role in metabolic disorders. Further studies are warranted to elucidate whether the altered DGK isoform expression profile in observed in obese insulin-resistant ob/ob mice has deleterious impact on tissue levels of PA and DAG as well as total DGK activity. Several DGK isoforms likely work in concert to modulate growth and metabolism in insulin sensitivity tissues.

Conflict of Interest
None declared.

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