Stanniocalcin-1 and -2 effects on glucose and lipid metabolism in white adipose tissue from fed and fasted rats

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Stanniocalcin-1 and -2 effects on glucose and lipid metabolism in white adipose tissue from fed and fasted rats

Elaine Sarapio, Samir K. De Souza, Jorge F.A. Model, Marcia Trapp, Roselis S.M. Da Silva.

Department of Physiology, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Correspondence: Departamento de Fisiologia; Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Sarmento Leite, 500, CEP: 90050-170, Porto Alegre, RS, Brazil.

E-mail address: elainesarapio@gmail.com (E. Sarapio).

Phone number.: +55 51 33083623
Abstract

Stanniocalcin-1 and -2 belong to a family of molecules that exhibit both paracrine and autocrine effects in mammalian cells. Human stanniocalcin-1 (hSTC-1) is expressed in a wide range of tissues, including white adipose tissue. In fed rats, hSTC-1 increases carbon flux from glucose to lipids in retroperitoneal white adipose tissue. Human stanniocalcin-2 (hSTC-2) is expressed in almost all tissues and regulates various biological processes. The aim of this work was to study the action of hSTC-1 and hSTC-2 in the lipid and glucose metabolism of epididymal white adipose tissue (eWAT) in rats in different nutritional states. This study shows for the first time an opposite effect of hSTC-1 and hSTC-2 on glyceride-glycerol generation from glucose in eWAT of fed rats. hSTC-1 stimulated the storage of triacylglycerol (TAG) in eWAT in the postprandial period, increasing glucose uptake and glyceride-glycerol generation from \(^{14}\)C-glucose. hSTC-2 decreased TAG synthesis, reducing glyceride-glycerol generation from \(^{14}\)C-glucose, direct phosphorylation of glycerol and fatty acid synthesis from \(^{14}\)C-glucose in eWAT of fed rats. However, both hormones increased glucose uptake in fed and fasting states. These findings provide evidence for a direct role of hSTC-1 and hSTC-2 in the regulation of lipid and glucose metabolism in eWAT of rats.

Key words: stanniocalcin 1, stanniocalcin 2, white adipose tissue, metabolism, fed, fasting, glucose, glycerol.
1. Introduction

Stanniocalcin (STC) was first isolated in fish where it was shown to regulate calcium/phosphate homeostasis (Chang et al. 1995; Olsen et al. 1996). Subsequently, two STC isoforms (STC-1 and STC-2) belonging to a family of hormones present in fish and mammals (Serlachius et al. 2004). In mammals, STC-1 is expressed in a wide range of tissues such as white adipose tissue (WAT) (Serlachius and Andersson 2004). The data indicate that mammalian STC-1 is produced and secreted by one cell type and is sequestered and acts in neighbouring cells, consistent with its paracrine/autocrine action (Yeung et al. 2012). Moreover, variants of high molecular weight, the big STCs, were described in the theca and interstitial cells of mouse and bovine ovary (Paciga et al. 2003), and in white adipose tissue of rats (Paciga et al. 2005).

The putative role of STC-1 in intermediary metabolism has been demonstrated in transgenic mice that overexpress this hormone, as these mice exhibit increased food and oxygen consumption in combination with reduced body weight (Filvaroff et al. 2002; Varghese et al. 1998). This energy-wasting phenotype is likely due to the stimulatory effects of STC-1 on mitochondrial electron transport (McCudden et al. 2004), which in excess can result in mitochondrial hypertrophy (Filvaroff et al. 2002). Recently, it was shown that hSTC-1 decreases the incorporation of $^{14}$C from glucose into total lipids in brown adipose tissue (BAT) of fed rats (Cozer et al. 2017). The presence of hSTC-1 in the incubation medium did not alter $^{14}$C-glucose and $^{14}$C-1-palmitic acid oxidation, or uncoupling protein 1 (UCP) expression in BAT of fed rats (Cozer et al. 2017). The colocalization patterns of STC-1 and perilipin in human white fat cells support the hypothesis of a central role of STC-1 in lipid metabolism (Serlachius et al. 2004; Serlachius and Andersson 2004).
Stanniocalcin-2 (STC-2) is a paralogue of STC-1 and in mammals is expressed in nearly all tissues and regulates various biological processes, such as ion transport, cell proliferation, reproduction, and the stress response (Yeung et al. 2012; Zeiger et al. 2011). Recently, Hjortebjerg et al. (2018) found high STC2 RNA levels in epididymal WAT (eWAT) and retroperitoneal WAT (rWAT) in mice, and Sarapio et al. (2019) found that Stc2 expression increased markedly in fasting rats BAT. López et al. (2017) proposed that STC2 is marker of the onset and progression of diabetes mellitus type 2. Moreover, Jiao et al. (2017) showed that STC-2 acts as an anorexic factor that leads to a significant reduction in body weight. Recently, Sarapio et al. (2019) showed that hSTC-2 reduced glycogen concentration in BAT from fed rats, which could lead to such deleterious effects on BAT as decreased thermogenic activity, esterification of FA and other adipocyte functions.

WAT is the largest energy reservoir of the body and it has been associated with metabolic regulation. This reserve depends on the balance of lipogenesis and lipolysis (Proença et al. 2014). Continuous supply of glycerol-3-phosphate (G3P) is necessary to TAG stock. A previous in vitro study from our laboratory demonstrated that, in rWAT of fed rats, hSTC-1 increases the incorporation of $^{14}$C from glucose into total lipids (Cozer et al. 2016). Actions of STC-1 and STC-2 on metabolism remain poorly understood in mammals, and our question is: Do these hormones control glucose and lipid metabolism in eWAT in fasted and fed rats? The present work is an in vitro investigation of the role of hSTC-1 and hSTC-2 in [2-$^{14}$C]-deoxy-glucose uptake, $^{14}$C-glyceride-glycerol generation from $^{14}$C-glucose or $^{14}$C-glycerol, and CO$_2$ production from $^{14}$C-glucose in eWAT of fed and fasted rats. In addition, insulin, leptin, and glucose levels were determined in the serum of rats.
2. Materials and Methods

2.1. Animals

Adult male Wistar rats weighing 300 ± 50g were kept at 22 ± 2°C with a light/dark cycle of 12 h/12 h. The rats were acclimated to animal facilities for one week and randomly divided into two groups: 1) fed rats consumed a standard diet (20% protein, 55% carbohydrate, and 4.5% lipids; Rodent Chow, Nutrilab®, São Paulo, Brazil) and water ad libitum; and 2) fasted rats were housed in individual cages, with a bed of wood shavings (to improve thermal comfort), and fasted for 48h with access to water ad libitum. The experiments were performed between 8:00 and 10:00 AM. The experimental protocol was approved by the official animal ethical committee (#27534) of the Universidade Federal do Rio Grande do Sul, Brazil. Animal use was in accordance with the Guide for the Care and Use of Laboratory Animals (The National Academies Press, Washington, D.C., Eighth Edition, 2011).

2.2. In vitro experiments

Rats were euthanized and blood samples were immediately collected into tubes containing a clot activator agent. Following centrifugation at 1000×g for 10 min, serum was separated and stored at -80°C prior to analysis. The eWAT was rapidly excised and sliced on an ice-cold dish containing Krebs Ringer Bicarbonate (KRB) buffer, pH 7.4. eWAT slices of 100±10 mg (500 mm thick) from fed or fasting (48h) rats were distributed between the control (without hormones) and experimental groups (with hSTC-1 and hSTC-2). In mammals, STC-1 and STC-2 exhibit paracrine, autocrine, and intracrine functions (Yeung et al. 2012); thus, in this study, we chose to slice the eWAT because cellular isolation would compromise the communication between cells. In all experiments, the slices were pre-incubated for 15 min at 37°C without hormones in
sealed flasks in 1 mL KRB pH 7.4 buffer with 1% phenylmethylsulfonyl fluoride (PMSF), equilibrated with O\textsubscript{2}:CO\textsubscript{2} (95:5, v/v). Pre-incubation and incubation were performed in the Dubnoff metabolic incubator under constant shaking at 37°C. The radioactivity measurement was performed in an LKB-Wallac liquid scintillation spectrometer.

2.3. Hormones

Human STC-1 and STC-2 were purchased from Ray Biotech Inc. (USA) and the concentrations used in this study (0.386; 3.86 or 38.6 pM) were previous employed to demonstrate the hSTC-1 effects on renal gluconeogenesis and glucose flux in rat BAT and rWAT (Cozer et al. 2017, 2016; Schein et al. 2015). To compare the effects of hSTC-1 and hSTC-2 we used the same concentrations for both hormones.

2.4. \textsuperscript{14}C 2-Deoxy-glucose uptake

Glucose uptake was determined according to Kaiser de Souza et al. (2013). Following the pre-incubation period, eWAT slices were removed from the medium, dried, and divided into two experimental groups: a) control group of fed or fasting (48h) rats: eWAT slices (100 ± 10mg) were incubated in 1 mL KRB pH 7.4 buffer with 1% bovine serum albumin (BSA) and 1% PMSF, equilibrated with O\textsubscript{2}:CO\textsubscript{2} (95:5, v/v) without hSTC-1 or hSTC-2, but in the presence of 0.2 μCi of [2-\textsuperscript{14}C]-deoxy-glucose (2-\textsuperscript{14}C-DG) (286 mCi/mmol, Perkin Elmer); b) hormone groups of fed or fasting (48h) rats: eWAT slices (100 ± 12mg) were incubated as described above but in presence of 0.386, 3.86 or 38.6 pM hSTC-1 or hSTC-2 plus 0.2 μCi of 2-\textsuperscript{14}C-DG. Following 1 h of incubation, the tissue slices were removed from the media, rinsed twice in cold KRB pH 7.4 buffers, blotted on filter paper, and transferred to screw cap tubes containing 1 mL
of distilled water. Subsequently, the tubes were alternately frozen and boiled three times. Aliquots (100 μL) of this solution and of the incubation media were used for radioactive counting in 2 mL of liquid scintillation cocktails (SLC): toluene- Triton X-100 (2:1, v/v) PPO 0.4%–POPOP 0.01%. Results are expressed as tissue/medium (T/M) ratio, i.e., dpm/mL tissue fluid divided by dpm/mL incubation medium.

2.5. Conversion of [U-14C]-glucose to 14CO2

Following pre-incubation, eWAT (100±15 mg) slices were incubated under the conditions described in Section 2.2 and 2.4, but in the presence of 0.2 μCi of [U-14C]-glucose (289 mCi/mmol; Perkin Elmer) and 5 mM of unlabelled glucose. The flasks contained small glass wells inside that were attached to the rubber caps and above the level of the incubation medium, containing small strips of Whatman™ 3MM paper and 1 M Hyamine® hydroxide (0.2 mL) to trap the 14CO2 produced. Incubation was stopped by adding 0.5 mL of 50% trichloroacetic acid through the rubber cap. Subsequently, the glass wells were transferred to vials containing 2 mL of SLC (Bueno et al. 1994). Values of 14CO2 production are expressed as mmol of 14C glucose incorporated into 14CO2, mg-1 of tissue, h-1 of incubation.

2.6. Incorporation of [U-14C]-glucose or [U-14C]-glycerol into 14C-glyceride-glycerol and 14C-fatty acid

Following pre-incubation, eWAT (100±17 mg) slices were incubated under the conditions described in section 2.2 and 2.4, but in the presence of 0.2 μCi of [U-14C]-glucose (289 mCi/mmol; Perkin Elmer), with 2 mM of unlabelled glucose or 0.2 μCi [U-14C]-glycerol (142 mCi/mmol; Perkin Elmer) plus 1 mM of unlabelled glycerol. After the incubation period, lipids were extracted from the eWAT slices with
chloroform:methanol (2:1, v/v), as described by Folch et al. (1957). Aliquots of the total lipid extracts were saponified with ethanolic KOH for 1 h at 70°C. Next, the samples were centrifuged twice with petroleum ether (5 min, 420×g). The fatty acid (FA) saponification reaction was completed with bromocresol green (0.04% U.B.L.) and the lower phase was acidified with 6N H₂SO₄ and centrifuged (5 min, 420×g) with 5 mL chloroform. Next, an aliquot of the water phase containing ¹⁴C-glyceride-glycerol was transferred to vials containing 5 mL SLC (Botion et al. 1995). The results were expressed in mmol of ¹⁴C-glucose or ¹⁴C-glycerol incorporated into ¹⁴C-glyceride-glycerol or ¹⁴C-fatty acid. mg⁻¹. h⁻¹ of incubation.

2.7. Biochemical analysis

Serum glucose concentration was determined by the glucose-oxidase method (Labtest Corporation, Minas Gerais, Brazil) and expressed as mg/dL of serum. Leptin and insulin levels in serum were determined with an enzyme-linked immunosorbent assay (ELISA) kit (Rat/Mouse Insulin Elisa Kit- EZRMI-13K and Rat Leptin EZRL-83K; Millipore Corporation, Massachusetts, USA). All samples were performed in duplicate. Leptin and insulin concentrations were expressed as ng/mL of serum.

Serum glucose, insulin and leptin levels were measured to confirm the metabolic status of the animal and to know exactly what the animal's plasma pattern was when the tissue was removed.

2.8. Statistical analysis

Data were expressed as the mean ± standard error of the mean or median (interquartile range), as appropriate. Data were verified for normality (Kolmogorov–Smirnov test) and homoscedasticity (Levene’s test). Two-way analysis of variance with
the post-hoc Tukey test was used for pairwise comparison of means. The Kruskal–Wallis test was used for comparisons of variables with skewed distribution with the post-hoc Dunn’s test. Unpaired Student’s t-test was used to compare differences in serum and eWAT weights of fed and fasted groups. All tests and comparisons with P<0.05 were considered statistically significant. Statistical analyses were performed using Prism Plot version 6.0 (GraphPad Prism Software) for windows and PASW Statistics version 18.0.

Results

In the fed state, hSTC-1 at 0.386 and 38.6pM increased (P<0.05) glucose uptake in eWAT slices (Figure 1A). In fasted (48h) rats, hSTC-1 at all tested concentrations markedly increased (P<0.05) glucose uptake in eWAT slices (Figure 1A). In fed rats, hSTC-2 increased (P<0.05) glucose uptake in eWAT slices at 3.86pM (Figure 1B). At all tested concentrations of hSTC-2, glucose uptake increased (P<0.05) approximately 43% in eWAT slices of fasted rats (Figure 1B).

In the control groups, no difference was observed in $^{14}$C incorporation from glucose into $^{14}$CO$_2$ between fed and fasted rat eWAT slices (Figure 2A and B). hSTC-1 and hSTC-2 did not significantly alter $^{14}$CO$_2$ formation from $^{14}$C-glucose in both fed and fasted animals (Figure 2A and B).

In eWAT of fed rats, 3.86 pM of hSTC-1 increased ($F_{(3, 58)}=5.839$, P=0.001) $^{14}$C incorporation from $^{14}$C-glucose into $^{14}$C-glyceride-glycerol compared with the control fed group (Figure 3A). In contrast, 38.6 pM of hSTC-2 decreased $^{14}$C-glyceride-glycerol formation (Kruskal–Wallis and Dunn’s tests, P<0.0001) from $^{14}$C-glucose in fed rat eWAT compared with the control group (Figure 3C). Fasting (48h) decreased ($F_{(1, 58)}=585.5$, P=0.0001) $^{14}$C-glyceride-glycerol formation from $^{14}$C-glucose in eWAT
compared with fed rats (Figure 3A and C). Moreover, no significant variations (P>0.05) in \(^{14}\text{C}\)-glyceride-glycerol formation from \(^{14}\text{C}\)-glucose were found in eWAT from fasting groups in the presence of both hSTC-1 and hSTC-2 (Figure 3A and C).

\(^{14}\text{C}\)-fatty acid synthesis from \(^{14}\text{C}\)-glucose was significantly decreased (F\(_{(1,64)}=148.3, P<0.0001\)) in control fasted rat eWAT slices compared with fed animals (Figure 3B and D). hSTC-1 (F\(_{(1, 64)}=4.457, P =0.006\)) and hSTC-2 (Kruskal–Wallis and Dunn’s tests, P<0.0001) at 38.6 pM markedly decreased \(^{14}\text{C}\)-fatty acid formation from \(^{14}\text{C}\)-glucose in eWAT of fed animals compared with the control group (Figure 3B and D). However, in fasted rats, hSTC-1 or hSTC-2 did not alter (P>0.05) \(^{14}\text{C}\)-fatty acid formation from \(^{14}\text{C}\)-glucose in eWAT (Figure 3B and D).

hSTC-1 did not significantly alter (P>0.05) \(^{14}\text{C}\) glycerol incorporation into glyceride-glycerol in eWAT slices of fed rats. However, 38.6 pM of hSTC-2 decreased (P<0.05) direct phosphorylation of \(^{14}\text{C}\)-glycerol by approximately50% in eWAT from fed rats (Figure 4C). Fasting (48h) markedly decreased (F\(_{(1, 54)}=117.4, P<0.0001\)) \(^{14}\text{C}\)-glycerol phosphorylation, and hSTC-1 and hSTC-2 did not change \(^{14}\text{C}\)-glycerol incorporation into \(^{14}\text{C}\)-glyceride-glycerol (Figure 4A and C). Both fasting and stanniocalcins did not significantly (P>0.05) affect fatty acid synthesis from \(^{14}\text{C}\)-glycerol in eWAT (Figure 4 B and D).

The eWAT weight decreased 42% (P<0.0001) after 48h fasting. Moreover, 48h fasting markedly decreased insulin (P<0.0001), glucose, and leptin serum levels (P<0.05) (Table 1).

**Discussion**

The present study demonstrates for the first time the participation of hSTC1 and hSTC2 in the regulation of glucose uptake and glyceride-glycerol-generation from \(^{14}\text{C}\)-
glucose and \(^{14}\)C-glycerol pathways in eWAT of fed and fasting rats. A major metabolic function of adipose tissue is the storage of energy in the form of TAG (Proença et al. 2014). To store triglycerides, adipocyte metabolism is flexible and tightly influenced by energy balance, hormonal factors, and the sympathetic nervous system (Frasson et al. 2012; Proença et al. 2014). In this study, food was available to fed rats until the experiments were conducted, and glucose, insulin, and leptin levels in the blood of these animals were elevated compared with the fasting state. In fed endocrine/metabolic pattern, both stanniocalcins increased glucose uptake in eWAT. Jiao et al. (2017) showed that in fed leptin-deficient (ob/ob) mice, systemic administration of STC-2 for 10 days decreased the blood glucose level with a marked improvement in insulin sensitivity. Thus, hSTC-1 and hSTC-2 could be new signalling elements for the regulation of blood glucose levels in the postprandial period by increasing glucose uptake in eWAT. Recently, Sarapio et al. (2019) found that hSTC-1 also increased glucose uptake in BAT from fed rats. Moreover, Schein et al. (2015) demonstrated that hSTC-1 decreased renal gluconeogenic activity in rats, suggesting the participation of these hormones in blood glucose homeostasis in mammals. So, the decrease in glucose synthesis by renal gluconeogenesis and the increase in glucose uptake in eWAT and BAT by STCs hormones would be an important contribution for glucose homeostasis in mammals, especially in type-2 diabetes mellitus patients.

On the other hand, hSTC-1 and hSTC-2 also stimulated glucose uptake in eWAT after 48h of fasting, despite low levels of serum insulin and glucose. Fasting-dependent decrease in Glut4 translocation from intracellular compartments to the cell surface in adipocytes and reduced use of glucose both \textit{in vivo} and \textit{in vitro} are well established and have been attributed to low levels of plasma insulin (Frasson et al. 2012; Proença et al. 2014). Thus, the effect of hSTC-1 and hSTC-2 on eWAT glucose uptake in the fasting
state can be the result of this hormone's action in the upregulation of Glut1, which is responsible for supplying cells with their basal glucose requirement (Barron et al. 2016; Yan 2017). STC-1 and STC-2 play an important role in the cellular response to stress, and 48h of fasting is a stressful situation for rats (Zhang et al. 2000; Zeiger et al. 2011). Glut1 mRNA and protein expression in 3T3-L1 adipocytes was increased 7-fold after 72h of glucose deprivation compared with the control group (Tordjman et al. 1990). In other stress conditions, such as cancer, STC-2 is highly expressed in these cells compared with normal tissues. Glut1 expression is often upregulated during oncogenesis, increasing the basal glucose uptake, which is likely essential for tumour growth (Warburg effect) (Barron et al. 2016; Thorens and Mueckler 2010; Yan 2017).

However, the effect of hSTC-1 and hSTC-2 on 1 and 4 Gluts expression and on insulin signalling pathway in the eWAT from fed and fasting (48h) rats will be necessary to clarify the increase in glucose uptake in these nutritional states.

Fasting for 48h decreased glyceride-glycerol production and de novo FA synthesis in eWAT, but hSTC-1, at the concentrations tested in this study, did not affect these pathways. In contrast, in fed state, hSTC-1 increased $^{14}$C incorporation from glucose into glyceride-glycerol. Also in rWAT from fed rats, hSTC-1 increased $^{14}$C incorporation from glucose into total lipids, but this effect was obtained with a hormone concentration ten times lower (0.386 pM) than that used in this study (Cozer et al. 2016). Thus, in the fed state, hSTC-1 maintains an adequate supply of G3P needed for FA esterification and TAG storage in rat eWAT. FA can be obtained through uptake from external sources (chylomicrons and very low-density lipoproteins) or by de novo FA synthesis from non-lipid substrates (Proença et al. 2014). Our data suggest that an hSTC-1-dependent decrease in the de novo FA synthesis from $^{14}$C-glucose would lead to an increase in the uptake of fatty acids from circulating lipoproteins, regulating blood
levels in the postprandial period. The storage of FA-TAG in WAT has been noted as a protective mechanism against the lipotoxicity of FA to other organs (Cao et al. 2008).

Although glycerokinase (GK) activity in WAT is relatively low compared with that in BAT and the liver (Kawashita et al. 2002; Martins-Santos et al. 2007), enzyme activity is regulated in this tissue: a marked decrease in WAT GK activity has been observed in fasted (48h) and diabetic rats (Frasson et al. 2012). Our data are in agreement with literature reports, which show a reduction in the capacity of direct phosphorylation of glycerol in WAT of rats fasting for 48h (Frasson et al. 2012). However, hSTC-1 did not affect direct phosphorylation of glycerol in eWAT of fed or fasting rats.

Conversely, the data of the present study show that hSTC-2 reduced the generation of glyceride-glycerol from glucose via dihydroxyacetone in the glycolytic pathway and the de novo synthesis of FA from glucose, but increased glucose uptake in eWAT of fed rats. Furthermore, hSTC-2 decreased the direct phosphorylation of glycerol in eWAT, but did not affect FA synthesis from glycerol. Thus, hSTC-2 is blocking the lipogenic effect characteristic of the postprandial period, when there is plenty of substrate, and the energy reserves must be increased in the WAT, which can be considered a waste of energy reserve induced by hSTC-2. These findings are consistent with the observation that intraperitoneal administration of STC-2 for 5 days in C57BL/6 mice decreased body weight and eWAT percentage (Jiao et al. 2017). The authors of this study also showed that intraperitoneal administration of STC-2 for 10 days in ob/ob mice markedly decreased mRNA levels of the adipogenic genes PPARγ, C/EBPα, and C/EBPβ (Jiao et al. 2017). Zeiger et al. (2011) suggest that STC-2 interacts with the endoplasmic reticulum calcium sensor STIM1 (stromal interaction molecule), controlling the store-operated calcium entry (SOCE), decreasing the
intracellular calcium concentration. Recently, studies indicated that mitochondria-associated membranes (MAMs) integrity was important for insulin signaling in both the liver and skeletal muscle and that MAM disruption is associated with hepatic and muscle insulin resistance, impacting glucose homeostasis (Tubbs et al. 2014). In this study the plasma insulin concentration in rats was higher (1.15 ± 0.25 ng/mL) in fed state. Therefore, reduction in the intracellular calcium concentration, induced by hSTC-2, could disrupt the signal transduction of insulin in this tissue, consequently decreasing TG synthesis during the postprandial period. Adipose tissue metabolism plays an important role in the regulation of energy balance and a rupture in its capacity of TG synthesis in a period of abundance of energetic substrates (fed state) will lead to a disruption in metabolic homeostasis such as decrease in energetic reserves, hormonal production and other adipocyte functions (Proença et al. 2014). This energy-wasting is found in transgenic mice that overexpress STC-2, as these animals exhibit increased food intake and oxygen consumption in combination with pre- and post-natal growth restriction, suggesting a metabolic defect induced by an excess of circulating STC-2 (Gagliardi et al. 2005). Another example of the negative metabolic effect of hSTC-2 is the decrease in the glycogen reserves in BAT of fed rats (Sarapio et al. 2019). On the other hand, the data showed that hSTC-2 not affect the pathways for TAG synthesis studied in this work.

Our data show that stanniocalcins play an important role in glucose uptake in eWAT in fed and fasting rats. This is surprising because no increase in glyceride-glycerol or de novo FA synthesis occurred in eWAT from fasting rats incubated in presence of hSTC-1 and 2. Moreover, hSTC-2 markedly raised glucose uptake in eWAT from fed rats, but it decreased glyceride-glycerol formation. A likelier explanation is that stanniocalcins increase lactate production from glucose in eWAT. In
vitro and in vivo studies in rat and human adipocytes showed that even in presence of oxygen the glycolytic pathway produces and releases large amounts of lactate (Jansson et al. 1994; Sabater et al. 2014). Thus, WAT conversion of glucose to lactate in fed rats would help lower blood glucose levels in the postabsortive period, a process potentially important in preserving insulin sensitivity by tissues (Sabater et al. 2014; Keuper et al. 2014). Fasting leads to alterations in the pattern of glucose metabolism in adipocytes, which increase lactate production from glucose to meet the body’s metabolic needs, e.g. as substrate for hepatic gluconeogenesis or oxidation in metabolic tissues (Digiloramo et al. 1992). Recently, Petersen et al. (2017) showed that MCT1 (SLC16A1) and MCT4 (SLC16A3) – the predominant lactate uptake and efflux pathways – are strongly upregulated at the mRNA and protein level upon adipocyte differentiation and after cold stimulation.

It is noteworthy that hSTC-1 and hSTC-2 did not increase $^{14}$C-glucose oxidation in eWAT in either the fed or fasting state. On the other hand, hSTC-1 increased $^{14}$CO$_2$ production from $^{14}$C-glucose by 85% in rWAT of fed rats (Cozer et al. 2016). The distinct responses of adipose tissue when submitted to the same stimulus can be attributed to the different roles and hormonal control of these depots in the energy metabolism of the body: while eWAT seems to be involved in local metabolic control, rWAT seems to be the main adipose depot responsible for the maintenance of energy homeostasis of the organism as a whole (Dos Santos et al. 2012; Ferreira et al. 2017).

This study shows for the first time an opposite effect of hSTC-1 and hSTC-2 on glyceride-glycerol production from glucose and direct phosphorylation of glycerol in eWAT of fed rats. Human STC-1 in the postprandial period (fed state) increased glyceride-glycerol formation from glucose. On the other hand, hSTC-2 decreased TAG synthesis, reducing glyceride-glycerol generation from glucose, direct phosphorylation
of glycerol, and FA synthesis from glucose in eWAT of fed rats. However, both hormones increased eWAT glucose uptake in the fed and fasting states. These findings provide evidence of a direct role of hSTC-1 and hSTC-2 in the regulation of lipid and glucose metabolism in eWAT from fed and fasting rats. Nevertheless, to complement the role of STCs in eWAT lipid metabolism, two important goals would be pursued further in vitro studies: 1) Effects of STC hormones on Glut 1 and 4 protein expressions and RNA levels after incubation of eWAT from fed and fasting rats in presence of different hSTC-1 or hSTC-2 and/or insulin concentrations; 2) Effects of different concentrations of hSTC-1 or hSTC-2 on insulin receptor substrate 1 (IRS-1) and protein kinase B (AKT) mRNA levels in eWAT from fed and fasting rats.

Acknowledgments

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**Table 1.** Epididymal white adipose tissue weight, glucose, insulin and leptin serum concentrations.

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<td>1.50 ± 0.11</td>
<td>123.9 ± 2.55</td>
<td>1.15 ± 0.25</td>
<td>8.52 ± 0.163</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Fasted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.87 ± 0.07**</td>
<td>74.77 ± 2.38*</td>
<td>0.33 ± 0.014**</td>
<td>2.16 ± 0.19*</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>

The results are presented as mean ± standard error of the mean. *P<0.0001, fed vs. fasted group (fasted effect). **P<0.05, fed vs. fasted group (fasted effect). eWAT, epididymal white adipose tissue.
Fig. 1 Effect of hSTC-1 (A) and hSTC-2 (B) on [2-14C]-deoxy-glucose uptake in eWAT of fed and fasted rats. Boxes indicate medians and 10th and 90th percentiles, \( n = 7-18; \) \( ^{a}P<0.05, \) hSTC vs. control group (hormone effect) in fed state; \( ^{b}P<0.05, \) hSTC vs. control group (hormone effect) in fasted state. eWAT, epididymal white adipose tissue; hSTC-1, human stanniocalcin-1; hSTC-2, human stanniocalcin-2.

Fig. 2 Effect of hSTC-1 (A) and hSTC-2 (B) on 14C-glucose incorporation into 14CO2 in eWAT of fed and fasted rats. Boxes indicate medians and 10th and 90th percentiles, \( n = 8-10; ^{*}P<0.05, \) fed vs. fasted state (fasted effect). eWAT, epididymal white adipose tissue; hSTC-1, human stanniocalcin-1; hSTC-2, human stanniocalcin-2.

Fig. 3 Effect of hSTC-1 and hSTC-2 on 14C-glucose incorporation into glyceride-glycerol (A and C) and fatty acid (B and D) in eWAT of fed and fasted rats. The bars represent means ± standard error of the mean (SEM). Boxes indicate medians and 5th and 95th percentiles, \( n = 7-12; ^{*}P<0.05, \) fed vs. fasted rats (fasted effect); \( ^{a}P<0.0001, \) hSTC vs. control groups (hormone effect) in fed state; eWAT, epididymal white adipose tissue; hSTC-1, human stanniocalcin-1; hSTC-2, human stanniocalcin-2.

Fig. 4 Effect of hSTC-1 and hSTC-2 on 14C incorporation from glycerol into glyceride-glycerol (A and C) and fatty acid (B and D) in eWAT of fed and fasted rats. The bars represent means ± SEM. \( n = 6-8. ^{*}P<0.001, \) fed vs. fasted rats (fasted effect); \( ^{a}P<0.0001, \) hSTC-2 vs. control groups (hormone effect) in fed state; eWAT, epididymal white adipose tissue; hSTC-1, human stanniocalcin-1; hSTC-2, human stanniocalcin-2.
Figure 1

A  hSTC-1

B  hSTC-2

141x263mm (300 x 300 DPI)
Figure 2

A

hSTC-1

Incorporation U-14C Glucose into CO₂ (nmol/mg·h)

Fed  Fasted

Control  0.38 nM  3.86 nM  38.6 nM

B

hSTC-2

Incorporation U-14C Glucose into CO₂ (nmol/mg·h)

Control  0.38 nM  3.86 nM  38.6 nM
Figure 3

A: hSTC-1

B: hSTC-1

C: hSTC-2

D: hSTC-2

Incorporation of 14C from Glucose into Glucose-Glycerol (mmol/mg)

Incorporation of 14C from Glucose into Fatty Acid (mmol/mg)

Control 0.50 μM 3.0 μM 10.0 μM

Fed   Fasted

199x180mm (300 x 300 DPI)