Exogenous thyroxine improves glucose intolerance in insulin-resistant rats

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Abstract

Both hypothyroidism and hyperthyroidism are associated with glucose intolerance, calling into question the contribution of thyroid hormones (TH) on glucose regulation. TH analogues and derivatives may be effective treatment options for glucose intolerance and insulin resistance (IR), but their potential glucoregulatory effects during conditions of impaired metabolism are not well described. To assess the effects of thyroxine (T4) on glucose intolerance in a model of insulin resistance, an oral glucose tolerance test (oGTT) was performed on three groups of rats (n=8): (1) lean, Long Evans Tokushima Otsuka (LETO), (2) obese, Otsuka Long Evans Tokushima Fatty (OLETF) and (3) OLETF + T4 (8.0 µg/100 g BM/day × 5 weeks). T4 attenuated glucose intolerance by 15% and decreased IR index (IRI) by 34% in T4-treated OLETF compared to untreated OLETF despite a 31% decrease in muscle Glut4 mRNA expression. T4 increased the mRNA expressions of muscle monocarboxylate transporter 10 (Mct10), deiodinase type 2 (Di2), sirtuin 1 (Sirt1) and uncoupling protein 2 (Ucp2) by 1.8-, 2.2-, 2.7- and 1.4-fold, respectively, compared to OLETF. Activation of AMP-activated protein kinase (AMPK) and insulin receptor were not significantly altered suggesting that the improvements in glucose intolerance and IR were independent of enhanced insulin-mediated signaling. The results suggest that T4 treatment increased the influx of T4 in skeletal muscle and, with an increase of Di2, increased the availability of the biologically active T3 to upregulate key factors such SIRT1 and UCP2 involved in cellular metabolism and glucose homeostasis.

Introduction

Thyroid hormones (TH) have a multitude of physiological effects related to thermogenesis, metabolism, heart rate and body composition (Araujo et al. 2008, Lin & Sun 2011). Through the transcriptional regulation of specific genes, THs have critical roles in the maintenance of glucose homeostasis (Chidakel et al. 2005, Villicev et al. 2007, Brenta 2010). However, evidence suggests that TH may induce non-genomic effects that contribute to cellular metabolism (Weitzel et al. 2001, Davis & Davis 2003). Although it is clearly established that TH drives metabolism, there remains conflicting evidence in the literature on the mechanisms involved in the regulation of glucose homeostasis (Medina et al. 2011, Aguer & Harper 2012). Both hyperthyroidism and hypothyroidism have been associated with complications in insulin signaling and glucose intolerance, a paradox that is...
likely associated with differential effects of TH on various tissues (Brenta 2010, Teixeira et al. 2012). Nonetheless, the literature suggests that synergies among T₃, glucose and lipid metabolism exist (Kim et al. 2002, Lin & Sun 2011). Exogenous T₃ induces insulin-stimulated glucose transport and glycolysis in the muscle (Moreno et al. 2011). Furthermore, T₃ potentiates insulin signaling, insulin sensitivity and an increase in insulin synthesis (Lin & Sun 2011). However, links among thyroid hormones, hyperglycemia and insulin resistance remain elusive.

In peripheral tissues, the genomic effects of TH occur after the intracellular transport of the predominate TH, thyroxine (T₄) and its deiodination to triiodothyronine (T₃), by either deiodinase type 1 (D1I) or type II (D1I2). The activity of these enzymes predominately found in skeletal muscle regulates the availability of T₃, and in turn, may indirectly regulate insulin signaling and glucose homeostasis. The binding of T₃ to its nuclear thyroid hormone receptor, THRβ-1, induces an energetically expensive and relatively time-consuming, transcriptional signaling cascade (Bernal & Refetoff 1977, Weitzel et al. 2001, Chidakel et al. 2005, Müller et al. 2014). One of the genes activated in this process, uncoupling protein 2 (Ucp2), may contribute to mitochondrial ATP production and to glucose homeostasis (Toda & Diano 2014). THs also promote metabolic changes through non-genomic effects, which can be manifested within minutes (as opposed to the longer genomic effects) (Weitzel et al. 2001). These non-genomic actions are independent of nuclear uptake of TH and may involve plasma membrane, mitochondrial or cytoplasm receptors that mediate transcriptional actions (Davis & Davis 2003). The monocarboxylate transporters, MCT8 and MCT10, are specific for THs and facilitate their transport in and out of the cell (Müller et al. 2014). Specifically, MCT10 has wide tissue distribution and can rapidly transport T₄, potentiating non-genomic effects (Van Der Deur et al. 2010).

Additionally, THs may also regulate sirtuin 1 (SIRT1), a NAD+-dependent deacetylase, that is involved in glucose homeostasis and insulin secretion. Recent studies have demonstrated that increasing expression of SIRT1 improves insulin secretion and sensitivity, especially in insulin resistance conditions (Moynihan et al. 2005, Sun et al. 2007). However, the direct effects of T₄ on muscle SIRT1 during insulin resistance are not well defined. Furthermore, downregulation or knockdown of Sirt1 induces insulin resistance in cells and tissues (Sun et al. 2007). SIRT1 may also interact with the TH receptor, THRβ1, which suggests a non-coincident relationship between SIRT1 and THs (Thakran et al. 2013).

Incongruences in the literature on the relationship between TH and glucose homeostasis during insulin resistance conditions exist. Furthermore, the simultaneous contributions of exogenous T₄ on UCP2 and SIRT1 to improved glucose tolerance are not well defined. To address the effects of TH on glucose intolerance, we chronically infused insulin-resistant, Otsuka Long Evans Tokushima Fatty (OLETF) rats with exogenous T₄ (OLETF+T₄).

**Materials and methods**

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committees of both Kagawa Medical University (Kagawa, Japan) and the University of California Merced (Merced, CA, USA).

**Animals**

Male lean (265±7 g), strain-control Long Evans Tokushima Otsuka (LETO) rats and obese (356±4 g) Otsuka Long Evans Tokushima Fatty (OLETF) rats (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan), both 9 weeks of age, were assigned to the following groups (n=8/group): (1) untreated LETO, (2) untreated OLETF and (3) OLETF+T₄ (8.0 µg/100 g BM/day×5 weeks). Rats were housed in groups of two or three in a specific pathogen-free facility (at Kagawa Medical University, Japan) under controlled temperature (23°C) and humidity (55%) with a 12-h light and darkness cycle. Animals were provided water and food *ad libitum*.

**T₄ administration**

Osmotic minipumps (Alzet, model 2006, Durect Corp., Cupertino, CA, USA) loaded with T₄ (Sigma-Aldrich) dissolved in 6.5 mM NaOH and 50% propylene glycol, were implanted subcutaneously, delivering a predefined dose (Klieverik et al. 2008, 2009).

**Body mass and food intake**

Body mass (BM) and food intake were measured daily. Sum of cumulative changes in body mass (ΣΔBM) was determined as the sum of consecutive changes in mean daily BM. Changes in ΣΔBM were determined by comparing slopes of the linear regressions for each group.
Blood pressure

Systolic blood pressure (SBP) was measured weekly in conscious rats by tail-cuff plethysmography (BP-98A; Softron Co., Tokyo, Japan) (Rodriguez et al. 2012, Vázquez-Medina et al. 2013).

Rectal temperature

Rectal temperature was measured weekly until the end of the study using a small animal warmer and thermometer (BWT-100A; Bio Research Center, Nagoya, Japan).

Oral glucose tolerance test

A week before the end of the study, all animals were fasted overnight (ca. 12 h) and oGTts performed as previously described (Rodriguez et al. 2012). The glucose area under the curve (AUC\textsubscript{glucose}) and insulin area under the curve (AUC\textsubscript{insulin}) were calculated by the trapezoidal method and used to calculate the insulin-resistant index (IRI) as previously described (Habibi et al. 2008).

Dissections

At the end of the treatment, all animals were fasted overnight (ca. 12 h) and tissues were harvested the subsequent morning. Trunk blood was immediately collected in chilled vials containing 50 mM EDTA and protease inhibitor cocktail. Samples were centrifuged (3000 \textit{g}, 15 min, 4°C), plasma was collected in cryovials and snap-frozen in liquid nitrogen. The heart, soleus muscle and epididymal (epi) and retroperitoneal (retro) fat were rapidly dissected, weighed and frozen in liquid nitrogen. All samples were stored at −80°C until further analysis.

Plasma analysis

Plasma insulin was measured using a commercially available kit (Rat Insulin ELISA kit; Shibayagi, Gunma, Japan) (Rodriguez et al. 2012). Plasma concentrations of total T\textsubscript{4}, total T\textsubscript{3} and serum concentrations of free T\textsubscript{4} were determined using commercial radioimmunoassay (RIA) kits (Coat-A-Count kit; Siemens Healthcare Diagnostics). Plasma thyroid-stimulating hormone (TSH) was determined with a commercially available rat ELISA kit (ALPCO Diagnostics, Salem, NH, USA). Plasma glucose, triglycerides (TG) and nonesterified fatty acids (NEFA) were measured as previously described (Rodriguez et al. 2012).

All samples were analyzed in duplicate with percent coefficients of variability of <10%.

Real-time quantitative PCR

The mRNA expressions of muscle Di1, Di2, Glut4, Mct10, Sirt1, Thrb1, and Ucp2 were quantified by real-time PCR as previously described (Vázquez-Medina et al. 2013, Martinez et al. 2016). Values were normalized for the expression of β-actin. Samples were run, with positive
Thyroxine improves glucose intolerance

Research

and negative controls, on a 7500 Real-Time PCR system (Applied Biosystems) in a 20µL reaction containing: 10µL of SYBR Green PCR Master Mix (Applied Biosystems), 6µL water, 0.5µL of each primer (20µmol/L) and 3µL of cDNA (150ng of RNA sample) (Vázquez-Medina et al. 2013, Martinez et al. 2016). Relative quantity of mRNA levels was plotted as fold-increase compared with the control group levels using the $2^{-\Delta\Delta Ct}$ method. Primer sequences used are provided in Supplementary Table 1 (see section on supplementary data given at the end of this article).

Quantification of protein expression by Western blot

Muscle protein content was assessed by Western blot as previously described (Rodriguez et al. 2012, Vázquez-Medina et al. 2013, Martinez et al. 2016). Membranes were incubated with primary antibodies against AMPKα (1:500, Cell Signaling Technology), phospho-AMPKα (Thr172) (1:350, Cell Signaling Technology), insulin receptor-β (1:500, Santa Cruz Biotechnology), phospho-IGF-I receptor-β (1:500, Cell Signaling Technology) and Na+/K+-ATPase-α (1:1000, Santa Cruz Biotechnology). Membranes were washed and incubated with secondary antibodies IR Dye 680RD donkey anti-mouse, IR Dye 680 RD donkey anti-rabbit or IR Dye 800CW donkey anti-rabbit (1:20,000) antibody. Proteins were then visualized using the LI-COR Odyssey Infrared Imaging System and quantified with ImageJ (NIH). In addition, consistency in loading equivalent amounts of total protein was confirmed and normalized by correcting for the densitometry values of Ponceau S staining (Gilda & Gomes 2013).

Statistics

Means (±s.e.) were compared by ANOVA followed by Fisher’s protected least-significant difference post hoc test and considered significant at $P<0.05$.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Relative masses (g/100g BM) of heart, soleus muscle and epi and retro fat in LETO, OLETF and OLETF + T4 after 5-week treatment (n=8/group).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LETO</td>
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<tr>
<td>Relative heart mass</td>
<td>0.38±0.03</td>
</tr>
<tr>
<td>Relative soleus muscle mass</td>
<td>0.05±0.008</td>
</tr>
<tr>
<td>Relative epi fat mass</td>
<td>0.94±0.06</td>
</tr>
<tr>
<td>Relative retro fat mass</td>
<td>1.3±0.07</td>
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$^\dagger P<0.05$ vs LETO.

Glucose tolerance was determined by comparing mean AUC values obtained from the oGTT. All statistical analyses were performed with the SYSTAT 11.0 software (SPSS).

Figure 2

Mean (±s.e.) (A) blood glucose and (B) plasma insulin levels taken during an oral glucose tolerance test and corresponding area under the curve (AUC) and (C) insulin resistance index (IRI) of LETO, OLETF and OLETF + T4. $^\dagger P<0.05$ vs LETO. $^\# P<0.05$ vs OLETF.
**Results**

**T₄ did not alter food consumption or body composition**

Daily food consumption and body mass and end-of-study tissue masses were measured to assess the potential effects of infused T₄ on body composition and food intake. Mean food intake was higher in OLETF than that in LETO (27.6 ± 0.3 vs 19.4 ± 0.3 g/day), but there was no significant treatment effect (27.4 ± 0.4 g/day) (Fig. 1A). BM increased by 25% in untreated OLETF compared to LETO, but T₄ treatment had no significant effect (Fig. 1B). Although the slope for the ΣΔBM regression for LETO (3.0 ± 0.13) was lesser than that for OLETF groups, no significant treatment effect was observed (4.4 ± 0.19 vs 4.1 ± 0.16) (Fig. 1C). Although relative heart and soleus masses were not different among the groups, the fat masses (epi and retro) were greater in the OLETF groups than those in the LETO; however, T₄ treatment had no additional effect (Table 1).

**T₄ did not alter SBP or core-body temperature**

SBP was measured to determine if improvements in metabolic defects translated to improvements in arterial blood pressure. Mean SBP in obese OLETF increased by 15% compared to lean LETO (138 ± 2 vs 117 ± 1 mmHG), and treatment with T₄ had no additional effect (140 ± 2 mmHG). These results demonstrate the development of elevated arterial blood pressure in the insulin-resistant phenotype, and the lack of improvement with the improved metabolic phenotype associated with the exogenous T₄ infusion. Rectal temperatures were also measured to determine the potential thermogenic effects of infused T₄. There were no consistent strains or treatment effects on core body temperature suggesting that the T₄-mediated cellular effects had none to minimal thermogenic effects (Supplementary Fig. 1).

**T₄ improved glucose intolerance and IRI**

An oGTT was conducted to determine the benefits of T₄ treatment on glucose intolerance and IRI during an
insulin-resistant condition. Mean AUC_{glucose} increased by 55% in OLETF compared to LETO and decreased 15% in T_4-treated OLETF (Fig. 2A). Mean AUC_{insulin} increased by 68% in OLETF compared to LETO and decreased 24% in T_4-treated OLETF (Fig. 2B). The IRI increased 86% in OLETF compared to LETO and decreased 34% in T_4-treated OLETF (Fig. 2C). These results suggest that T_4 improved glucose intolerance, insulin resistance and IRI in the OLETF rat.

The obese, insulin-resistant phenotype is associated with hyperthyroidism

TSH, total T_4, free T_4 and total T_3 were measured to determine the thyroid hormone profile of the insulin-resistant animals. TSH was 15% greater in OLETF compared to LETO and was reduced 8% with T_4 treatment (Fig. 3A). Total T_4, free T_4 and total T_3 were consistently greater in OLETF compared to LETO (Fig. 3B, C and D). In addition, fT4 and tT4 levels were exacerbated with the infusion of T_4 as expected, confirming the successful infusion of exogenous T_4. The elevated fT4 levels increase the availability of biologically active hormone to support the increase in DI2 for conversion to intracellular T_3. To further demonstrate the cellular effectiveness of the T_4 infusion, Na^+-K^+ ATPase protein expression, a sensitive marker of T_4 activity, was measured. The protein expression of Na^+-K^+ ATPase increased nearly 3-fold in the treated OLETF group compared to its untreated counterpart (Fig. 4). A 50% reduction in protein expression was associated with the obese OLETF compared to the lean LETO (Fig. 4).

T_4 increases muscle mRNA expressions of Mct10, Di2, Ucp2 and Sirt1

To assess the effects of T_4 on the mRNA expressions of key regulators of TH-mediated signaling and metabolism in muscle, Mct10, Di1/2, Thrb1, Ucp2 and Sirt1 were measured. Muscle Mct10 mRNA expression decreased over 50% in untreated OLETF compared to LETO, and levels were completely recovered with T_4 (Fig. 5A). Although the decreases in Di2 with OLETF did not reach significance, Di1 levels were significantly reduced (Fig. 5B and C) and Di2 levels nearly doubled with T_4 (Fig. 5C). No strain or treatment effect was detected in Thrb1 expression (Fig. 5D). Although no strain effect in Ucp2 and Sirt1 expression levels was detected, T_4 increased levels 40% (Fig. 5E) and 2.7-fold (Fig. 5F), respectively.

T_4 improvements in IRI are independent in static changes in insulin signaling

The phosphorylation of AMPK and insulin receptor was measured to help determine the contributing factors to the improvements in glucose intolerance and IRI with T_4 infusion. No profound changes in phosphorylation of insulin receptor or AMPK were detected (Fig. 6A and B). Although mean AMPK phosphorylation decreased 30% with T_4 treatment, it did not reach significance (Fig. 6B). Glut4 mRNA expression did not exhibit a strain effect, but T_4 reduced levels by 31% (Fig. 6C). Collectively, these data suggest that the improvements in glucose clearance and IRI are independent of static enhancements in...
Thyroxine improves glucose intolerance

\[ \text{Na}^{+}-\text{K}^{+}\text{ATPase is a downstream target of T}_4 \text{ that is responsible for an electrochemical gradient that provides energy for the transport of many ions, metabolites and nutrients such as glucose (Forst et al. 2000). The upregulation of Na}^{+}-\text{K}^{+}\text{ATPase also imposes an energetic burden on cellular metabolism. Typically, diabetes-induced metabolic changes are also associated with alteration to the Na}^{+}-\text{K}^{+}\text{ATPase activity. For example, impaired skeletal muscle glucose uptake in high-fat diet, insulin-resistant Wistar rats was associated with a reduction in Na}^{+}-\text{K}^{+}\text{ATPase and increased T}_3 \text{ increased its activity (Lei et al. 2004, Ho 2011). In the present study, the muscle content of Na}^{+}-\text{K}^{+}\text{ATPase was statically lower in the OLETF rat, and exogenous T}_4 \text{ more than completely recovered the suppressed protein content suggesting that the T}_4 \text{ infusion was effective at inducing a cellular response. Furthermore, the increase in muscle Na}^{+}-\text{K}^{+}\text{ATPase likely contributed to the improvement in IRI in the T}_4\text{-treated group by increasing cellular metabolism independent of insulin and AMPK signaling. Additionally, increased Na}^{+}-\text{K}^{+}\text{ATPase activity is associated with increased SIRT1 (Yuan et al. 2014), similar to the relationship demonstrated in the present study. Thus, increasing cellular metabolism independent of enhanced insulin signaling may produce greater benefits in ameliorating obesity-associated insulin resistance than directly targeting insulin-mediated mechanisms.}

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The transport of T4 into the cell is required regardless of whether the cellular outcomes are genomic or non-genomic. Although originally the transport of TH was thought to be passive, multiple membrane-bound transporters have been recently identified including MCT8 and MCT10 suggesting that TH-mediated effects may be regulated by the presence or upregulation of these specific transporters. Thus, the concentration of thyroid hormone within the cell will determine the rate of cellular regulation of TH-mediated signaling (Van Der Deure et al. 2010). In the present study, Mct10 was reduced and associated with elevated plasma levels of T4 in insulin-resistant OLETF rats suggesting that the elevated plasma levels are attributed to reduced cellular transport, and consequently contribute to the impairment of the HPT axis. Furthermore, the reduced Mct10 levels may also explain the decreasing trends in the mRNA expression levels of Di1 and Di2 as reduced availability of T4 for conversion to T3 reduces the demand for deiodinases. This is corroborated by reduced protein content of Na+–K+ ATPase, a surrogate marker of energetic demand and downstream target of TH signaling. Conversely, exogenous T4 reversed these strain-associated defects.

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\text{T}_4 \text{ does not improve the strain-associated effect on lipid metabolism}
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Fasting plasma TG and NEFA were measured to assess the effects of T4 treatment on lipid metabolism. Fasting plasma TG (85 ± 9 vs 36 ± 5 mg/dL) and NEFA (0.78 ± 0.09 vs 0.45 ± 0.04 mEq/L) increased in OLETF compared with LETO. However, circulating lipids (79.1 ± 11.0 mg/dL and 0.67 ± 0.01 mEq/L) were not significantly altered with T4.

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Hyperthyroidism and hypothyroidism are both associated with insulin resistance suggesting that thyroid hormones may have little if any effect on the regulation of glucose during insulin resistance. In the present study, we investigated the role that exogenous T4 has on glucose intolerance and insulin signaling in a model of insulin resistance. We demonstrated that the infusion of T4 significantly improved the insulin-resistant condition and glucose intolerance in the insulin-resistant OLETF rat.

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and likely contributed to the improvements in glucose intolerance and IRI. The recovery of the Mct10 expression levels, although not associated with a decrease in plasma T₄ due to the constant infusion, was associated with increased DI2 and Na⁺-K⁺ ATPase. Increased expression of the transporter should translate to a greater availability of T₄ inside the cell, and with increased DI2 expression (and likely activity given the increase in fT4), there would be increased conversion to the more biologically active T₃. This is supported by the complete recovery of muscle Na⁺-K⁺ ATPase.

With skeletal muscle accounting for about 40–50% of the total body mass, it is the largest contributor to resting energy expenditure and insulin-induced glucose utilization (Marsili et al. 2010). Furthermore, DI2 has been shown to have a greater effect on T₃-dependent gene transcription than DI1 (Maia et al. 2005). Subjects with genetic defects to DI2 have reduced glucose turnover and DI2-knockout mice become insulin resistant (Maia et al. 2005, Hong et al. 2013). The present study would corroborate that further demonstrating that exogenous T₃ is effective at restoring this insulin resistance-associated defect in DI1/2. In addition, DI2 has been shown to provide a protective effect against diet-induced obesity-associated glucose dysregulation (Marsili et al. 2011), which would explain the increase in mRNA of Di2 in our treated, diet-induced, obese insulin-resistant OLETF rats. The increased mRNA expression of the transporter and DI2 can be linked with a higher availability of the T₃ inside the cell to potentiate the increases of downstream targets that contributed to the improvement in glucose intolerance and insulin resistance.

In muscle, the genomic effects of T₃ are mediated by its receptor, THrβ1. Furthermore, T₃ can increase the expression of THrβ1, which may activate (phosphorylate) AMPK, a key regulator of cellular metabolism (Cantó & Auwerx 2013, Wang et al. 2014). When active, AMPK triggers the translocation of GLUT4 to the plasma membrane, to increase the uptake of glucose (Cantó & Auwerx 2013). GLUT4 is an insulin-regulated protein that contributes to whole-body glucose homeostasis, and therefore, is a key target for understanding insulin resistance in type 2 diabetes mellitus (T2DM) (Teixeira et al. 2012). However, in this present study, there was no detectable increase in the static activations of muscle insulin receptor or AMPK and a reduction of mRNA expression of Glut4 suggesting that the improvements in insulin resistance with infused T₄ was independent of static enhancements in the traditional insulin-mediated signaling pathway. Alternatively, the improvements may have been accomplished through the contributions of other glucose transporters not measured here. The lack of an increase in Thrb1 would also suggest that the improvements in glucose tolerance were either mediated through: (1) non-genomic mechanisms such as increased cellular metabolism and/or (2) genomic effects that did not necessitate static elevations in THrβ1 expression. That is, the increase in T₃ transport (via increased MCT10) and deiodination (via increased DI2) in the T₃-infused rats was likely sufficient to maintain the levels of THrβ1 expression to facilitate the increase in cellular metabolism that translated to improved glucose tolerance. Furthermore, T₃ can rapidly increase glucose uptake independent of increased GLUT4 at the cell surface (Teixeira et al. 2012), thus, the increase in DI2 likely increased the cellular levels of T₃ to facilitate glucose uptake independent of GLUT4.

SIRT1, a downstream target of AMPK, is thought to be nutritionally regulated, and low levels have been reported with high-fat and hyperglycemic conditions (Brandon et al. 2015). AMPK typically regulates Sirt1 expression via NAD⁺ content; however, studies have shown that phosphorylation of AMPK had no effect on SIRT1 levels when knocked out or overexpressed. Furthermore, T₃ stimulation enhanced the transcription of genes involved in mitochondrial fatty acid oxidation and gluconeogenesis via upregulation of SIRT1 (Thakran et al. 2013, Brandon et al. 2015). This is consistent with our findings that demonstrated a decrease in Sirt1 in OLETF rats that were completely recovered by the infusion of T₄. Furthermore, the increase in muscle SIRT1 expression with T₄ treatment was likely independent of AMPK activation. Thus, the improvements in insulin resistance in the treated group may be the result of non-TH-mediated genomic effects mediated in part by SIRT1. This is supported by a study demonstrating that SIRT1 can directly bind DNA-bound transcription factors, including nuclear receptors, and influence transcription (Suh et al. 2013).

UCP2 is also a downstream target of THrβ1. Although the mechanisms describing UCP2 regulation of glucose metabolism is not well understood, an important link between the two is apparent. For example, subjects with a polymorphism in the transcription of Ucp2 are associated with obesity and T2DM (Toda & Diano 2014). In addition, UCP2 increases glucose-stimulated insulin secretion in pancreatic beta cells (Toda & Diano 2014). The insulin resistance phenotype in OLETF is associated with an approximately 20% decrease in hepatic UCP2 protein content, which is more than completely restored with ARB treatment and associated with improved IRI (Montez et al. 2012). Similarly,
the present study demonstrates a substantial increase in muscle Ucp2 expression with infused T₄ and an associated improvement in IRI suggesting that the maintenance and/or restoration of systemic UCP2 is an important factor in abating the manifestation and consequences of insulin resistance. Additionally, as with the current study, the previous improvements in IRI were independent of static improvements in the insulin signaling cascade suggesting that maintenance and/or restoration of UCP2 contributes to improvement in IRI independent of direct insulin-mediated signaling (Rodriguez et al. 2012). Furthermore, the observed improvements in glucose metabolism may be attributed to the upregulation of UCP1 in brown adipose tissue (BAT). BAT in rodents has been shown to improve not only the control of thermogenesis but also glucose metabolism (Matsen et al. 2013). Furthermore, a human case study demonstrated that T₄ treatment induced brown adipose tissue and ameliorated diabetes in a patient with extreme insulin resistance. This improvement was associated with an increase of UCP1 and DI2 in BAT (Skarulis et al. 2010).

A major concern of clinical hyperthyroidism is the deleterious effects associated with potential thyrotoxicosis (Villicev et al. 2007, Araujo et al. 2008, Brenta 2010). Exogenous T₄ treatment did not have any deleterious effects on the body composition, food consumption, excessive thermogenesis and SBP suggesting that thyrotoxicosis was not a compounding factor. As expected, exogenous T₄ administration decreased TSH levels and increased plasma T₄, indicative of intact negative feedback mechanism and successful accomplishment of chronic elevation of circulating T₄ with the infusion. The untreated OLETF was also characterized by relative hyperthyroidism with elevated triglycerides and non-esterified fatty acids suggesting that thyroid hormones do not profoundly improve the dyslipidemia associated with insulin resistance. Furthermore, the OLETF model presents with an impaired hypothalamic–pituitary–thyroid negative feedback mechanism, as the nearly doubled T₄ levels are associated with a 15% increase in circulating TSH. This impaired negative feedback is restored in the T₄-infused rats. Additionally, hyperthyroid states may be associated with elevated plasma free fatty acids and glucose levels, which possibly contribute to the decrease in insulin sensitivity during this condition. However, it remains unclear if this condition is a cause or a consequence of insulin resistance in hyperthyroidism (Teixeira et al. 2012).

In conclusion, we found that exogenous T₄ treatment improved glucose intolerance and insulin resistance via increased cellular metabolism. The increase in cellular metabolism was achieved by increases in the mRNA expressions of Mct10, Di2, Sirt1 and Ucp2 that likely resulted in increased content of muscle Na⁺–K⁺ ATPase. The results suggest that T₄ treatment increased the influx of T₄ in skeletal muscle and, with an increase of DI2, increased the availability of the biologically active T₃ to upregulate key factors such SIRT1 and UCP2 involved in cellular metabolism and glucose homeostasis. Furthermore, these changes in cellular events are largely suppressed in the obese, insulin-resistant OLETF rat suggesting that these factors contribute to the manifestation of metabolic syndrome in this model. The ability of exogenous T₄ to ameliorate this condition is independent of the phosphorylation (activation) of the insulin receptor and AMPK, which probably translates to a reduced expression of Glut4. These results collectively suggest that impaired thyroid hormone regulation of key factors in cellular metabolism contributes to the glucose intolerance associated with insulin resistance and ultimately the development of metabolic syndrome.
Thyroxine improves glucose intolerance


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